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**Interspecies Extrapolations of Halocarbon Respiratory and Tissue Kinetics:
Applications to Predicting Toxicity in Different Species**

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Neurobehavioral depression was compared with uptake of PCE and TRI in the brain and blood of rats, and with TRI in mice. The direct measurements of halocarbon concentrations in exhaled breath, blood and tissues have provided an extensive data base that has been used to formulate and validate the physiologically-based pharmacokinetic (PBPK) models for exposure to halocarbons. The partition coefficients for developing PBPK models for rats and dogs were determined from detailed tissue determinations in these species. Using the direct measurements data, the ability of the PBPK models to generate accurate predictions of halocarbon concentrations were evaluated in blood, exhaled breath, and seven tissues.

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TECHNICAL SUMMARY

A series of experiments have been conducted to provide a pharmacokinetic data base for interspecies comparisons and for formulation and validation of physiologically-based pharmacokinetic models. The basic experimental design has involved giving equal doses of halocarbons in different species, including mice, rats, and dogs. Perchloroethylene (PCE), tetrachloroethane (TET), trichloroethylene (TCE), and trichloroethane (TRI) have been employed as test chemicals, in order to evaluate the relative importance of the physicochemical property of volatility on the kinetics and toxicity of halocarbons. In order to determine the dose received in target organs and other tissues, serial samples of brain, liver, kidney, lung, heart, skeletal muscle, and adipose tissue have been taken and analyzed for halocarbon content after administration of PCE, TET, and TRI in rats, and PCE and TET in dogs. For neurobehavioral studies, an operant testing system and rotorod system have been employed for monitoring the central nervous system effects of halocarbons. Neurobehavioral studies have been conducted following oral and inhalation exposure to PCE, and from inhalation exposure to TRI in rats. Neurobehavioral depression was compared with uptake of PCE and TRI in the brain and blood of rats, and with TRI in mice. The direct measurements of halocarbon concentrations in exhaled breath, blood and tissues have provided an extensive data base that has been used to formulate and validate the physiologically-based pharmacokinetic (PBPK) models for exposure to halocarbons. The partition coefficients for developing PBPK models for rats and dogs were determined from detailed tissue determinations in these species. Using the direct measurements data, the ability of the PBPK models to generate accurate predictions of halocarbon concentrations were evaluated in blood, exhaled breath, and seven tissues.

I. OVERALL OBJECTIVE AND SPECIFIC AIMS

The overall objective of this project is to investigate the scientific basis for the interspecies extrapolation of pharmacokinetic and neurobehavioral toxicity data. Direct measurements of the respiratory elimination and tissue concentrations of halocarbons over time in two species will be used to formulate and validate physiologically-based pharmacokinetic models for halocarbon exposure. These models will be used for: (a) prediction of the time-course of the respiratory elimination and target organ levels of halocarbons; (b) interspecies extrapolations (i.e., scale-up from smaller to larger laboratory animals and ultimately to man). A combined physiological pharmacokinetic-toxicodynamic model for halocarbon exposure will subsequently be developed and evaluated for its ability to predict neurobehavioral effects under specified exposure conditions.

SPECIFIC AIMS in the proposed studies are to:

- 1) Determine the respiratory elimination of physicochemically disparate volatile organic compounds (VOCs) in two animal species. Rats and dogs will be administered selected halocarbons by inhalation and oral exposure. Concentrations of expired parent compounds will be monitored in the exhaled breath for appropriate periods during and following exposure. Together with monitoring of the respiratory volumes of the test animals, this data will enable calculation of the cumulative uptake and elimination of the halocarbons. Data from both species will be compared to existing data sets for respiratory elimination in humans.
- 2) Delineate the tissue disposition of inhaled halocarbons in two animal species. Rats and dogs will be exposed to halocarbons by inhalation. Concentrations of the parent compound in brain, liver, heart, lung, kidney, skeletal muscle, and adipose tissue will be measured over time, in order to provide an assessment of the actual target organ dose for correlation with neurobehavioral toxicity and for development and validation of physiologically-based pharmacokinetic and toxicodynamic models.
- 3) Validate physiologically-based pharmacokinetic (PBPK) models for predicting the tissue pharmacokinetics of halocarbons in two animal species. PBPK models that have been initially developed in rats in our previous studies, with blood and expired air data for inhalation exposures and with tissue data for intraarterial and oral exposures, will be further validated for accuracy in interspecies extrapolations. Direct measurements of exhaled breath and tissue concentrations and associated parameters in rats and dogs in the initial two phases of the presently proposed project will be used to further test the accuracy of our PBPK models. Models will be developed each for the rat and the dog. The observed animal data and pharmacokinetic parameters will be used to formulate allometric relationships which can then be used to predict human disposition of halocarbons.
- 4) Correlate the neurobehavioral toxicity of inhaled VOCs in two species with the target organ concentration. Rats and dogs will be exposed to selected

halocarbons at defined inhaled concentrations and lengths of exposure. Neurobehavioral tests for operant performance will be performed periodically during and after exposures. The magnitude of central nervous system (CNS) effects of each solvent will be correlated with the target organ (i.e., brain) halocarbon concentration, as determined in (2), at each time-point. Thereby, it will be possible to determine whether equivalent target organ doses in the rats and dogs elicit CNS effects of comparable magnitude in each species.

- 5) Develop and validate toxicodynamic models for inhaled halocarbons. Brain halocarbon concentrations will be correlated with the magnitude of neurobehavioral toxicity in an appropriate equation. These relationships will be used in conjunction with the PBPK model developed in (3). The CNS effects observed in (4) will be compared to predicted effects to assess the validity of the model in the two species tested. Validated models may allow the prediction of CNS effects over time of exposure using: a) extrapolations from pharmacokinetic data; b) simulations in the absence of experimental data.

II. FUNDAMENTAL HYPOTHESIS TESTING

A very important question faced by scientists and administrators conducting risk assessments is the relevance of toxic effects seen in animals to anticipated adverse effects in humans. Pharmacokinetic studies are playing an increasingly important role in species to species extrapolations in toxicology. Blood concentration over time, as a measure of bioavailability, is routinely accepted as an index of the level of chemical in the entire system, and therefore a representative parameter of toxic effects. This assumption can be misleading, in that blood concentrations may not be reflective of the concentration of chemical or active metabolite at the local site of effect in a target organ or tissue. It is now recognized that chemical toxicity is a dynamic process, in which the degree and duration of toxic effect in each tissue is dependent upon systemic absorption, tissue distribution, interactions with cellular components, and clearance from the tissue and body by metabolism and excretion. Estimation of the risk of toxic injury from pharmacokinetic data is based on the assumption that the intensity of the response from a given dose is dependent upon the magnitude of the dose received by a target tissue. A related assumption can be stated in the form of a HYPOTHESIS:

THE DOSE RECEIVED IN A PARTICULAR TARGET TISSUE IN ONE SPECIES WILL HAVE THE SAME DEGREE OF EFFECT AS AN EQUIVALENT TARGET TISSUE DOSE IN A SECOND SPECIES.

There are surprisingly few scientific data that are applicable to this basic assumption, although it is a very important premise in interspecies extrapolations in toxicology. If valid, it is a logical basis on which to evaluate the suitability of different species as predictors of toxicity in humans (i.e., the species in which target organ deposition is most similar to man would likely be an appropriate animal model for toxicity testing).

A series of parallel studies in different animal species is therefore being employed to test the foregoing hypothesis. The rat and dog have been subjected

to equivalent exposure to halocarbons. Similarities and differences in respiratory elimination, tissue disposition, and toxicity between the species are being determined. Neurobehavioral alterations will be used as a toxic end-point in the currently proposed work, since: a) OSHA and EPA commonly use neurobehavioral effects as the basis for deriving standards for exposure to VOCs; b) central nervous system (CNS) depression is caused by and can be directly correlated with the concentration of parent compound in the CNS.

III. ANALYTICAL DETERMINATIONS OF HALOETHANES AND HALOETHENES IN TISSUES

A paper has been published in a scientific journal on the method that was developed for tissue determinations of aliphatic halocarbons. The reprint of this paper is included as section A of the appendix, and the reference is as follows:

Chen, X.M., Dallas, C.E., Muralidhara, S., Srivatsan, V., and Bruckner, J.V. "Analyses of volatile C₂ haloethanes and haloethenes in tissues: sample preparation and extraction." Journal of Chromatography 612: 199-208 (1993).

Characterization of the systemic uptake, distribution and elimination of volatile organic compounds (VOCs) requires reliable analytical techniques for measuring the concentration of the chemicals in different tissues of the body. An extraction procedure was developed which minimized loss of the readily volatilizable compounds, so that they could subsequently be quantified by headspace gas chromatography. The procedure was evaluated using four C₂ halocarbons [i.e., perchloroethylene (PCE), 1,1,1-trichloroethane (TRI), 1,1,2,2-tetrachloroethane (TET), and 1,1,2-trichloroethylene (TCE)] of varying physicochemical properties. Portions of 0.5 to 1 g of liver, kidney, brain, heart, lung, skeletal muscle, fat and blood from rats were spiked with PER to yield a theoretical concentration of 4 µg/g tissue. Two homogenization procedures were evaluated: (a) tissues were homogenized in saline, followed by isooctane extraction; and (b) tissues were homogenized in isooctane and saline (4:1, v:v). The latter approach resulted in a significantly higher percent recovery of PCE from most tissues. Neither homogenization nor the presence of saline affected PCE standards prepared in isooctane. It was observed that the volume of the aliquot of isooctane taken for PCE analysis was important, in that aliquots >25µl could not be used. PCE concentrations were determined in tissues or rats following in vivo (i.e. intraarterial administration) of the halocarbon using the latter (i.e., isooctane) homogenization approach. This approach was also employed to examine the efficiency of recovery of PCE, TET, TRI, and TCE from seven tissues and from blood. Percent recoveries of each of the four halocarbons ranged from 73-104% for the seven spiked tissues. The recoveries did not appear to be tissue-dependent, despite differences in homogenization time required for different tissues. Recovery, however, did vary somewhat with test chemical, with the least volatile, most lipophilic compounds exhibiting the highest recovery.

IV. UTILIZATION OF TISSUE CONCENTRATION-TIME COURSE DATA FOR PBPK MODEL DEVELOPMENT FOR PCE

Although knowledge of the deposition of chemicals in target tissues is of major importance in risk assessment, PBPK models have to date been used primarily to forecast uptake and elimination of VOCs from the bloodstream. Blood concentration over time, as a measure of bioavailability, is routinely accepted as an index of the level of chemical in the body, and therefore a representative index of toxic effects. This assumption can be misleading in that blood concentrations may not be reflective of the concentration of chemical or active metabolite at the local site of effect in a target tissue. Thus, a more logical measure of target organ exposure is the area under the tissue versus time curve for the reactive chemical. Relatively little has been published on the use and validation of PBPK models for prediction of time integrals of tissue exposure of VOCs, because of a paucity of tissue concentration versus time data sets. This lack of a data base is due to the considerable effort required in such studies and to technical problems with quantitation of the volatile chemicals in solid tissues.

The design of physiologically-based models incorporates anatomical and physiological characteristics of the test animal, as well as physicochemical properties and biochemical constants specific to the chemical being studied. Specific organs or groups of organs with common properties are characterized in regard to their intrinsic volumes and blood flow rates. Chemicals are defined in respect to their partition coefficients and metabolic rates. Since we are working with volatile chemicals, provision is made not only for metabolism but for pulmonary input and output. The modeling approach provides a relevant description of movement of a halocarbon through the body of the test animal, since it takes into account important factors which govern the pharmacokinetics of halocarbons.

The tissue disposition of perchloroethylene (PCE) was determined experimentally in rats in order to 1) obtain model input parameters from in vivo data for the development of physiologically based pharmacokinetic (PBPK) model, and 2) forecast the disposition of PCE in a variety of tissues following inhalation exposure. For the derivation of model input parameters, male Sprague-Dawley rats received a single bolus of 10 mg PCE/kg bw in polyethylene glycol 400 by intraarterial injection through an indwelling carotid arterial cannula. For the testing of the PBPK model simulations, male Sprague-Dawley rats inhaled 500 ppm PCE for 2 hours in dynamic exposure inhalation chambers. Serial samples of brain, liver, kidney, lung, heart, skeletal muscle, perirenal adipose tissue, and blood were taken for up to 48 hr following ia administration and during inhalation exposure and up to 72 hr post-exposure. Blood and tissue PCE concentrations were analyzed using a gas chromatography headspace technique. A PBPK model was developed using these parameters derived from ia data and used to make predictions of the tissue concentrations in each of the model compartments during and following PCE inhalation exposure. Overall, predicted tissue concentrations were in agreement with the direct measurements conducted over time in the sever tissues and in blood.

This study has been described in a manuscript that is now being submitted to the journal, Toxicology and Applied Pharmacology. The manuscript is included as Section B of the Appendix, and the citation is as follows:

Dallas, C.E., Chen, X.M., O'Barr, K., Muralidhara, S., Varkonyi, P., and Bruckner, J.V. "Development of a Physiologically Based Model for Perchloroethylene Pharmacokinetics Using Tissue- Concentration Time Data"

The use of tissue concentration time data was therefore shown to be of significant utility in the development of PBPK models. Model parameters estimation is aided for tissue:blood partition coefficients, as direct measurements can be used to determine these values for as many model compartments as is feasible in the experimental design. Determination of the reliability of the model predictions of tissue concentrations in each compartment using this approach could be useful in improving the accuracy of risk assessments related to target organ disposition of VOCs.

V. EVALUATION OF PBPK MODEL PREDICTIONS OF PCE UPTAKE AND RESPIRATORY ELIMINATION

The objective of this phase of the project is to evaluate the accuracy of the PBPK models, which allows prediction of the concentration of halocarbons in exhaled breath and tissues over time following inhalation exposure. The pharmacokinetic studies conducted in the project provide a unique data base from which to test the model. Data from the direct measurements of exhaled breath and blood concentrations have been compared to simulated values calculated from mass-balance differential equations comprising the model. Thereby, the accuracy of the model is being tested and adjustments made where necessary to improve the model simulations.

Toward this end, the pharmacokinetics of perchloroethylene (PCE) was studied in male Sprague-Dawley rats to characterize systemic uptake and respiratory elimination by direct measurements of the inhaled compound. Fifty or 500 ppm PCE was inhaled for 2 hr through a one-way breathing valve by unanesthetized male Sprague-Dawley rats of 325-375 g. Serial samples of separate inhaled and exhaled breath streams, as well as arterial blood, were collected during and following PCE inhalation and analyzed by headspace gas chromatography (GC).

The studies involved in the testing of the PBPK model have been included in a manuscript, which is also being submitted to Toxicology and Applied Pharmacology. The manuscript is included as Section C of the Appendix, and the citation is as follows:

Dallas, C.E., Muralidhara, S., Chen, X.M., Ramanathan, R., Varkonyi, P., Gallo, J.M., and Bruckner, J.V. "Characterization of Perchloroethylene Uptake and Respiratory Elimination for Evaluating Physiologically Based Model Predictions"

As part of the respiratory elimination studies to be conducted in the current proposal, respiratory volumes were directly measured. In addition to the calculation of cumulative uptake during exposure and elimination following exposure, monitored respiratory volumes were useful in providing accurate values for use in the PBPK models. Substantial variations in the magnitude of respiratory parameters can occur in the same species due to stress, age differences animal care procedures, variability in animals provided by different vendors, and differences in respiratory monitoring techniques in different laboratories. Thus, the use of published values for input into the PBPK models may lead to inaccurate predictions. The magnitude of the administered dose and quantity of compound exhaled during inhalation exposures are highly dependent on the rate and volumes of respiration since exhalation is the primary route of elimination for PER and TET.

Parameters for a blood flow limited physiologically-based model were determined from tissue concentration-time data in the manuscript in Appendix B, and a model characterized with PCE eliminated in the exhaled breath and to a limited extent by liver metabolism. PCE concentrations in the blood and exhaled breath were well predicted by the PBPK model.

The ability of the current PBPK model to predict disposition of PCE increases the confidence in the utility of the model in health risk assessments. The use of tissue concentration-time data for establishing PBPK model parameters was found to be helpful in developing a model to provide accurate predictions both during and following inhalation exposure.

VI. INTERSPECIES COMPARISONS IN PBPK MODEL PARAMETERS ESTIMATION

Similarities in the anatomy and physiology of mammalian species make interspecies extrapolations (i.e. animal scale-up) possible. Models developed in a series of species may be scaled based on allometric relations to allow prediction of chemical concentrations in other species without the collection of additional experimental data. Model parameters such as tissue volumes and blood flows can be scaled based on allometric relationships which are functions of animal weight. The ability to scale-up animal physiological models to humans is a powerful tool to obtain predictions of tissue chemical concentrations in humans.

One need in development of the PBPK model for use in different species are tissue-blood partition coefficients. Absorption rates, metabolism constants as well as other model input parameters, of course, these parameters are likely to vary considerably between species.

The tissue disposition of perchloroethylene (PCE) was determined experimentally in two mammalian species of markedly different size, in order to obtain model input parameters for in vivo data for the development of a physiologically based pharmacokinetic (PBPK) model to forecast the disposition of PCE in each species. Male Sprague-Dawley rats and male beagle dogs received a single bolus of 10 mg PCE/kg bw in polyethylene glycol 400 by gavage. Serial samples of brain, liver, kidney, lung, heart, skeletal muscle, perirenal adipose tissue, and blood were taken for up to 48 hr following PCE administration. Blood

and tissue PCE concentrations were analyzed using a gas chromatography headspace technique. While the blood:air partition coefficient was similar to that value determined using *in vitro* data in the literature, tissue:blood partition coefficients were 1.4 to 2.8 times greater for the *in vivo* data in this study compared to the available published *in vitro* values. The PCE blood:air partition coefficient for the dog was twice that of the rat, and the tissue:blood partition coefficients were 1.5 to 3.0 times greater in the rat relative to the dog.

In the development of these PBPK models, one of the most important model parameters is the tissue:blood partition coefficient. This value governs the rate of the transfer of the chemical between the blood and each of the tissue compartments represented by the model. An *in vivo* approach to deriving tissue:blood partition coefficients for PBPK models has been described, in which the tissue-concentration time course of the test chemical is employed. While this approach provides an *in vivo* description of the rate of transfer of the chemical for each tissue for which the data is available, there has been little opportunity for its use because of the paucity of detailed tissue concentration time data for most VOCs, including PCE. Therefore, the time course of uptake, disposition, and elimination of PCE in blood and seven tissues was determined in two species, and the data utilized in deriving partition coefficients for a PBPK model for PCE.

These investigations describing PBPK model estimation in rats and dogs for PCE are included in a manuscript. This paper is presented as section D of the Appendix, and the citation is as follows:

Dallas, C.E., Chen, X.M., Muralidhara, S., Varkonyi, P., Gallo, J.M., Tackett, R.L., and Bruckner, J.V. "Species Differences Between Rats and Dogs in Physiologically-Based Model Parameter Estimation Using Perchloroethylene Tissue Pharmacokinetics"

Where available, *in vivo* tissue concentration time data can help to evaluate *in vitro* data, contribute to the design of tissue compartments of PBPK models, and test important assumptions in interspecies scaling of the models.

VII. UTILITY OF PBPK MODEL PREDICTIONS BETWEEN SPECIES, DOSES, AND ADMINISTRATIVE ROUTE

In order to test how robust the PBPK model is at this point of development, it is useful to test the predictive ability under a variety of experimental conditions.

Pharmacokinetic models developed in a series of species may be scaled based on allometric relations to allow prediction of chemical concentrations in other species without the collection of additional experimental data. Model parameters such as tissue volumes and blood flows can be scaled based on allometric relationships which are functions of animal weight. Changing the input parameters for the concentration of chemical entering the exposed species allows for extrapolations between dose. Altering the point of entry of the chemical into the PBPK model enables pharmacokinetic comparisons between routes of administration using an otherwise similar modeling approach.

A primary objective of the current study was to evaluate the accuracy of a PBPK model in predicting blood levels of PCE between species, exposure concentration and route of administration in comparison to observed data from matched experiments conducted for that purpose. Where possible, equivalent doses were administered in two species of wide variation in size, the rat and the dog, and a variety of doses used in each species. These exposures were repeated using two routes of administration, and venous blood levels over time were used to document the time course of PCE uptake, disposition, and elimination under each of the experimental regimens. The utility of the PBPK model predictions were then evaluated under a variety of experimental conditions.

A manuscript concerning this data is now being prepared. The current version of this paper is included as section E of the Appendix, and the citation is as follows:

Dallas, C.E., Chen, X.M., Muralidhara, S., Varkonyi, P., Tackett, R.L., and Bruckner, J.V. "Comparisons Between Species, Doses, and Routes of Administration of Perchloroethylene Pharmacokinetics Using a Physiologically Based Pharmacokinetic Model"

It was demonstrated that the PBPK model is accurate in many aspects of the experimental conditions tested but some problems exist. Predicted concentrations in venous blood across a 10-fold range of doses for ia administration were accurate in rats. In dogs, however, there was some overprediction of PCE concentrations between the two doses tested.

VIII. PBPK MODEL PARAMETER ESTIMATION FOR TET USING TISSUE CONCENTRATION TIME COURSE DATA IN RATS AND DOGS

The PBPK model development completed for PCE in the two species was then extended to TET.

As a class of chemicals, halocarbons have low solubility in blood and high volatility (i.e., low blood:air partition coefficients), as well as rapid vascular-alveolar transfer. Thus, a substantial proportion of the blood's burden of halocarbons should be removed during each pass through the lungs. It follows that halocarbons with low blood:air partition coefficients should be more efficiently eliminated and have a less prolonged CNS depressant action, than halocarbons with relatively high partition coefficients. In order to test this PREMISE, tetrachloroethylene (PCE) and 1,1,2,2-tetrachloroethane (TET) were utilized. Both PCE and TET are poorly metabolized (Ikeda and Ohtsuji, 1972) and have similar oil:blood (i.e., fat/blood) partition coefficients, but PCE has a much lower blood:air partition coefficient.

The ability of the PBPK model to accurately predict both PCE and TET pharmacokinetics would demonstrate the capacity of the model for halocarbons of a wide variation in volatility.

The studies for TET PBPK model parameter estimation were presented at the 1993 meeting of the Society of Toxicology. The citation for the abstract is as follows:

Dallas, C.E., Chen, X.M., Muralidhara, S., Varkonyi, P., Tackett, R.L., Gallo, J.M., and Bruckner, J.V. "Determination of physiologically-based model partition coefficients from 1,1,2,2-tetrachloroethane (TET) tissue pharmacokinetics." 32nd Annual Meeting of the Society of Toxicology, New Orleans, LA; Toxicologist 13: 173, 1993.

In order to improve the accuracy of physiologically-based pharmacokinetic (PBPK) model predictions, tissue:blood partition coefficients were estimated using direct measurements of tetrachloroethane (TET) in rat and dog tissue and blood. Male Sprague-Dawley rats received a single bolus of 10 mg/kg of TET in polyethylene glycol by oral gavage or by intraarterial administration through an indwelling carotid arterial cannula. Male beagle dogs received a single intraarterial bolus of 10 mg/kg of TET. In order to establish a steady-state equilibrium in test animals, other male beagle dogs were given a loading dose of 5 mg/kg via a jugular vein for 4 hours.

Serial samples of brain, liver, kidney, lung, heart, skeletal muscle, adipose tissue, and blood were taken up to 24 hr following TET administration from the animals in each of these test groups. Percent recovery of TET from tissues was also determined for rats and dogs, using isolated tissues spiked with known concentrations of TET. Blood and tissue concentrations were analyzed for TET content using a GC-ECD headspace technique.

Percent recovery ranged from 87.8% from rat skeletal muscle to 95.7% from dog heart. There were no significant differences in % recovery between the two species. Considerably longer half-lives in the tissues and blood of dogs relative to an equivalent PCE administration in rats were found. Overall, values predicted by the PBPK model were in close agreement with measured values in the nonfat tissues. Following an equivalent dose the larger of the two test species had a larger area under the tissue concentration (AUC) curve for all tissue compartment except kidney than the smaller species. These results will be helpful in the further investigation of some of the uncertainties in the application of tissue dose metrics in interspecies extrapolations of TET using PBPK models.

Male beagle dogs (8-14 kg), obtained from Marshall Farms, and male Sprague-Dawley rats (325-375 g), obtained from Charles River Laboratories, were employed in these studies. For the animals intended to receive the ia dose, an indwelling carotid arterial cannula was surgically implanted the day before the exposure. The cannula exited the body of the test animal behind the head, and the animals were allowed to recover from anesthesia until the following day. For ia and po administration, TET was administered at a dose of 10 mg/kg as an emulsion in polyethylene glycol (PEG) in a single bolus dose through the carotid arterial cannula or by oral gavage.

Groups of 4 rats and 3 dogs were serially sacrificed (using etherization) following dosing at the following time intervals - 5, 15, 30, and 45 minutes, and

1, 1.5, 2, 3, 4, 12, 16, 20, 24, and 36 hours for rats and 0.5, 2, 4, 8, and 12 hours for dogs. Blood samples were obtained by cardiac puncture. For establishing steady-state equilibrium, dogs received a loading dose of 5 mg/kg through a jugular vein cannula. The animals then received a constant infusion of 28 μ g/min/kg through the cannula, and blood levels were monitored for TET over the next four hours. Once the blood levels remained constant for at least 2 hours, it was assumed that steady state had been achieved. Tissues isolated from unexposed animals were spiked with 0.1, 1, 5, 10, and 20 mg TET/g of tissue and the percent recovery for each tissue in each species established.

Approximately 1 gram each of liver, kidney, brain, lungs, heart, fat and muscles were quickly removed from all animals and placed in 4 ml cold saline. Tissues were homogenized for the shortest possible time intervals, specific for each organ, or reduce the volatilization of the test compound during homogenization. The TET in each sample was then extracted with 8 ml isooctane. A 20 μ l aliquot was placed in an 8 ml headspace vial, which was capped and subjected to controlled temperature and pressure conditions in a Perkin-Elmer HS-6 Headspace Sampler.

Analysis was made of the TET in the vial headspace on an OV-17 (6' x 1/8") stainless steel column in a Perkin Elmer gas chromatograph with an electron capture detector. The column temperatures were: detector-375°C, column-120°C, headspace-90°C, injector-200°C. Values were compared to a standard curve, and the tissue concentration corrected for the percent recovery characteristic for each tissue.

Due to the highly lipophilic nature of TET, the maximum tissue concentration (C_{max}) in the fat was substantially higher (ranging from 7-28 and 11-48 times greater for rats and dogs, respectively) than the other tissues. The $t_{1/2}$ and AUC was also much longer in the fat than in the other tissues for both species and both routes of administration. The AUC for each of the dog tissues was substantially greater than that of the rat tissues. The $t_{1/2}$ and C_{max} values were also greater for each tissue in the dog than in the rat. The elimination $t_{1/2}$ for the brain was longer than in all other non fat tissues in the dog, but was shorter than the other non fat tissues in the rat following an equivalent ia administration. The poorly perfused tissues (muscle) had the lowest AUC and C_{max} for both po and ia administration in the rat, but was higher than other non fat tissues in the dog.

In comparisons with observed tissue concentration-time data, tissue concentrations of TET were well predicted by the PBPK model in brain, blood, kidney, heart, and lung over the length of the time course following TET administration. Following po administration in rats and dogs following ia administration were well predicted. There was some overprediction of muscle concentrations of TET in the dog by the model. In both species and routes of administration, there was a tendency for underproduction of the TET concentration during part of the time course. Following an equivalent ia exposure, the underproduction occurred in the later time points for rats and in the earlier time points for the dog.

Partition coefficients determined from the AUC for dog tissues following ia administration of TET were similar to values calculated from the steady-state

determinations in tissues for brain, heart, and fat. There was more than a two-fold difference (2.0-2.8) between the partition coefficients for kidney, lung, and muscle calculated by the two approaches. Using the partition coefficients calculated using the AUC method for ia exposures, the PBPK model for TET was therefore demonstrated to have considerable utility in accurately predicting tissue levels in rats and dogs.

IX. EVALUATION OF THE ACCURACY OF PBPK MODEL PREDICTIONS FOR TET ACROSS DOSES, SPECIES, AND ROUTE OF ADMINISTRATION

An important consideration in health risk assessments of halocarbon solvents is the validity of species to species comparisons in the uptake, disposition, and elimination of the chemicals following ingestion. Therefore, the relative toxicokinetics between species of wide variation in size was evaluated following tetrachloroethane (TET) ingestion. Male Sprague-Dawley rats and beagle dogs were administered TET at dose of 10 or 30 mg/kg. The halocarbon was administered in polyethylene glycol (PEG) in a single bolus either orally (po), or by intraarterial administration (ia) through an indwelling carotid arterial cannula. Blood samples were collected from an indwelling cannula in the jugular vein at intervals up to 48 hours following administration, and the halocarbon concentrations analyzed by headspace gas chromatography. The terminal elimination half-lives of TET in dogs were significantly longer than in rats for both routes of administration and for both po doses. Bioavailability, peak blood levels and the area-under-the-blood-concentration-time curves were also higher in dogs relative to equivalent dose in rats po and ia. These results show that there are significant species differences in the toxicokinetics and bioavailability of ingested TET, and that further evaluations are needed for making interspecies comparisons of toxicokinetic data for halocarbons.

Male beagle dogs (5-10 kg), obtained from Marshall Farms, and male Sprague-Dawley rats (325-375 g), obtained from Charles River Laboratories, were employed in these studies. For the animals intended to receive ia administrations of the test compounds, an indwelling carotid arterial cannula was surgically implanted the day prior to the exposure. For procuring blood samples following halocarbon administration, an indwelling jugular vein cannula was implanted in all the test animals. Both cannulas exited the body of the test animal behind the head, and the animals were allowed to recover from anesthesia until the following day. Food was withheld during the 18 hr recovery period before dosing.

The rats and dogs were administered a single bolus dose of either 10 or 30 mg/kg TET, using polyethylene glycol (PEG) as a dosage vehicle. Both oral doses were administered using a gavage needle for rats and a teflon tube for dogs. The ia administration was conducted using the carotid arterial cannula. While rats exhibited no neurobehavioral effects following TET administration, the dogs receiving the 30 mg/kg ia dose demonstrated a very high degree of central nervous system (CNS) depression. Data for ia administration in dogs is therefore presented only for the 10 mg/kg dose.

Serial 20 μ l blood samples were taken at selected intervals for up to 48 hrs following dosing. The concentrations of TET in the blood samples were determined by headspace analysis using a Perkin-Elmer Sigma 300 gas chromatograph

equipped with an electron capture detector and an automatic headspace analyzer. The operating conditions for the 6-ft x 1/8-inch stainless steel column were: headspace sampler temperature, 100°C; injection port temperature 200°C; column temperature, 140°C; detector temperature, 400°C; column packing, OV-17; flow rate for argon/methane carrier gas, 60 ml/min.

The blood concentration-time data were evaluated by R-strip (Micromath Scientific Software) and Lagran (M. Rocci and W.J. Jusko) computer programs for the assessment of the appropriate pharmacokinetic model and calculation of relevant pharmacokinetic parameters.

PBPK model predictions for TET in rats and dogs can be found in the Appendix, Section G. PBPK model prediction of TET in the venous blood of rats were well predicted at the 10 mg/kg dose, but underpredicted over the course of the 30 mg/kg dose following both ia and po administration. Predictions of TET predictions in dogs following oral administration were accurate over most of the time course for both doses. Predictions of TET concentrations in dogs following ia administration were not well predicted, especially in the terminal elimination phase.

Absorption of TET following oral administration was very rapid in rats and dogs, with peak blood levels achieved in both species and at both doses between 12 and 22 minutes. The maximum concentration of TET reached in the blood was higher in dogs than in rats in all cases, though this difference was only statistically significant for intraarterial administration. The half-life of TET was longer in the blood of dogs than in rats, and at a high level of significance by intraarterial administration and at both doses given orally. Following both oral and intraarterial administration of TET, AUC was significantly higher in dogs than in rats. The bioavailability of TET from oral exposure was higher in dogs than in rats. Following equivalent ia doses of 30 mg/kg TET, dogs exhibited severe CNS depression (including unconsciousness) while rats demonstrated no appreciable neurobehavioral effects. No CNS effects were observed in either species following po administration at either dose.

X. EVALUATION OF NEUROTOXICITY OF PERCHLOROETHYLENE USING ROTAROD AND COMPARISON WITH BLOOD AND BRAIN LEVELS

The rotarod has been in use since the mid 1950's, primarily as a tool to evaluate the effects of neurotoxins. Since its introduction there have been a wide variety of modification to the basic concept of the rotarod test. The rotarod has been used by several researchers to evaluate the neurotoxicity of solvents. The purpose of this study was to measure the effects of PCE on the rotarod performance in dynamically exposed animals during testing, and to attempt to correlate performance on the rotarod to blood and brain concentrations of perchloroethylene.

A dowel of 1 1/4 inch diameter and approximately 40 inches in length was adapted to rotate 13" above the floor of Rochester type dynamic flow chamber. The rod was divided into three 7 inch compartments by 4 cardboard disks 13 inch in diameter. The rod was driven by an externally mounted, variable speed motor

supported outside of the chamber and attached directly to the rod via a side portal. For this experiment a speed of 10 rpm was used.

1,1,2,2 Perchloroethylene (PCE) (tetrachloroethylene) was obtained from Aldrich Chemical Company (Milwaukee, WI). Test atmospheres of PCE were generated by vaporization of the compound in different combinations of air flow and temperature control schedules. Approximately 1 liter of liquid chemical was placed into a glass dispersion flask. Nitrogen was passed through the dispersion flask and was directed into the chamber. A heating mantle was placed around the dispersion flask, and narrowly controlled temperature limits maintained for volatilization continuity. The entire halocarbon generation system was enclosed in a specially fabricated safety box. The box was maintained under constant negative pressure during inhalation exposures in the same manner as the inhalation chamber. Exhaust air from both the safety box and the inhalation chamber was vented to HEPA and charcoal filter, so that all chemical was scrubbed before release to the environment. Concentrations were monitored inside of the chamber by using a Foxboro Miran 1b ambient air analyzer.

Eight to 12 CD-1 male albino mice, weighing 35 to 40 grams were obtained from Charles River Laboratories (Raleigh, NC). All mice were maintained on constant light-dark cycle with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. Mice were housed in plastic cages, with tap water and Purina Mouse Chow #5010 provided ad libitum. Training sessions were begun after at least a 6 day acclimation period.

Animals would be placed on the rotarod and performance time monitored using an electronic stopwatch. If animals fell during the training session, their performance time would be noted and the animals were immediately placed back onto the rotarod. Training sessions for each animal would last a maximum of one hour per day. If the animal had successfully maintained itself on the rotarod for one hour, the animal was removed by the tail and returned to its housing. The animals were trained a total of six to eight days. After this training period, animals which produced a two day average performance of 30 minutes or less over the two days prior to exposure were removed from the study group. Only one animal was eliminated using this criteria. Most animals gave a two day base line performance of 40 to 60 minutes (Figure 1).

After 24 hours from the completion of training, a concentration of 1500 ppm, 2000 ppm, or 3000 ppm Perchloroethylene was generated and stabilized in the dynamic exposure chamber. The animal was placed on the rotarod via a portal door and performance was monitored using an electronic stopwatch. The animal was immediately removed from the chamber via the portal when it fell from the rotarod, and sacrificed using cervical dislocation. Approximately .2 ml blood was collected by cardiac puncture, and whole brain was removed from the animal. Both tissues were quickly placed into previously chilled 20 ml glass scintillation vials containing 4 ml of isooctane and 1 ml saline solution. Each tissue was homogenized with a Ultra-Turrax SDT homogenizer (Tekmar Co., Cincinnati, OH). Homogenization was accomplished as quickly as possible to minimize loss of the compound due to volatilization. The homogenates were then centrifuged at 1800 x gravity for 10 minutes at 4 degrees centigrade in capped scintillation vials. An aliquot of the isooctane layer was diluted with pure

isooctane and transferred to 20 ml headspace vials. These vials were capped with latex rubber septa and crimped to ensure an airtight seal.

Each sample vial was then placed into the autosampler unit of a Perkin Elmer 8500 Gas Chromatograph (Norwalk CT). The following conditions were maintained on the G.C.: column oven temperature, 90°C; injector temperature 120°C; detector temperature 120°C. The analysis was carried out using a 6' x 1/8" stainless steel detector column packed with 3% ov-17 (80-100 mesh) Carrier gas was argon/methane @60 ml/min. Data analysis was done using Lotus 1-2-3 with a standard curve run on everyday of analysis.

Each animal's performance time on the rotarod was compared to the 2 day baseline average from the last two days on training. Animals responded to the perchloroethylene exposures in a dose dependent manner (Figure 1). Blood and brain Perchloroethylene concentrations were averaged and compared with exposure levels (Figure 2).

Although this test did successfully generate a dose response curve with PCE, it is difficult to interpret blood and brain concentrations of PCE at the time of fall and performance on the Rotarod. There are some significant limitations with this test method. Another factor is the significant limitations with this test method. One factor is the significant amount of training required to attain the baseline performance time on the rotarod. From this dose response curve, there is little justification for extending exposure below the 1500 ppm mark or above the 3000 ppm mark. The accelerod has apparently produced a more sensitive testing with alcohol and acrylamide oral dosages in rats. However, the accelerod was not chosen because it would introduce an extra variable into the experimental schedule. We also are currently generating a pharmacokinetic model of Perchloroethylene in mice to confirm the blood and brain levels measured with the rotarod mice and to examine the possibility that performance on the rotarod could be related to other factors such as rate of uptake of compound into brain and subsequently the degree of neurological impairment.

Table 1

Animal Baseline Performance and number of days trained

<u>Two Day Baseline (min)</u>	<u>Number of Days Trained</u>
60	8
42	8
49	8
60	8
51	7
60	7
60	7
39	7
53	7
60	7
36	7
60	7
55	8
60	8
60	8
4	8
60	8
36	8
45	8
31	8
55	6
60	6
60	6
60	6
60	6
60	6
60	6
60	6
60	6
60	6
60	6
60	6

Figure 2— Performance on Rotarod as Percent Baseline Performance

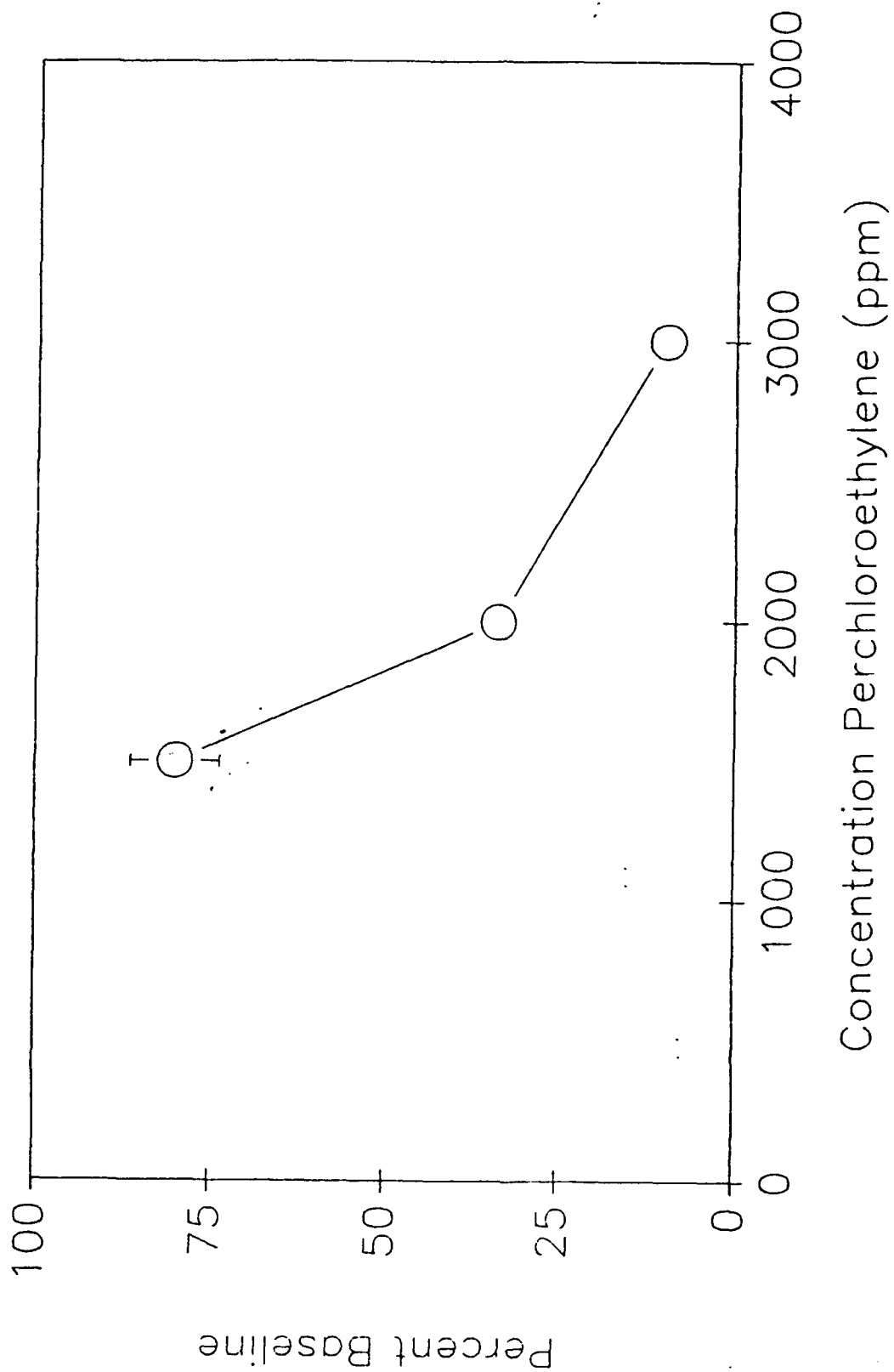
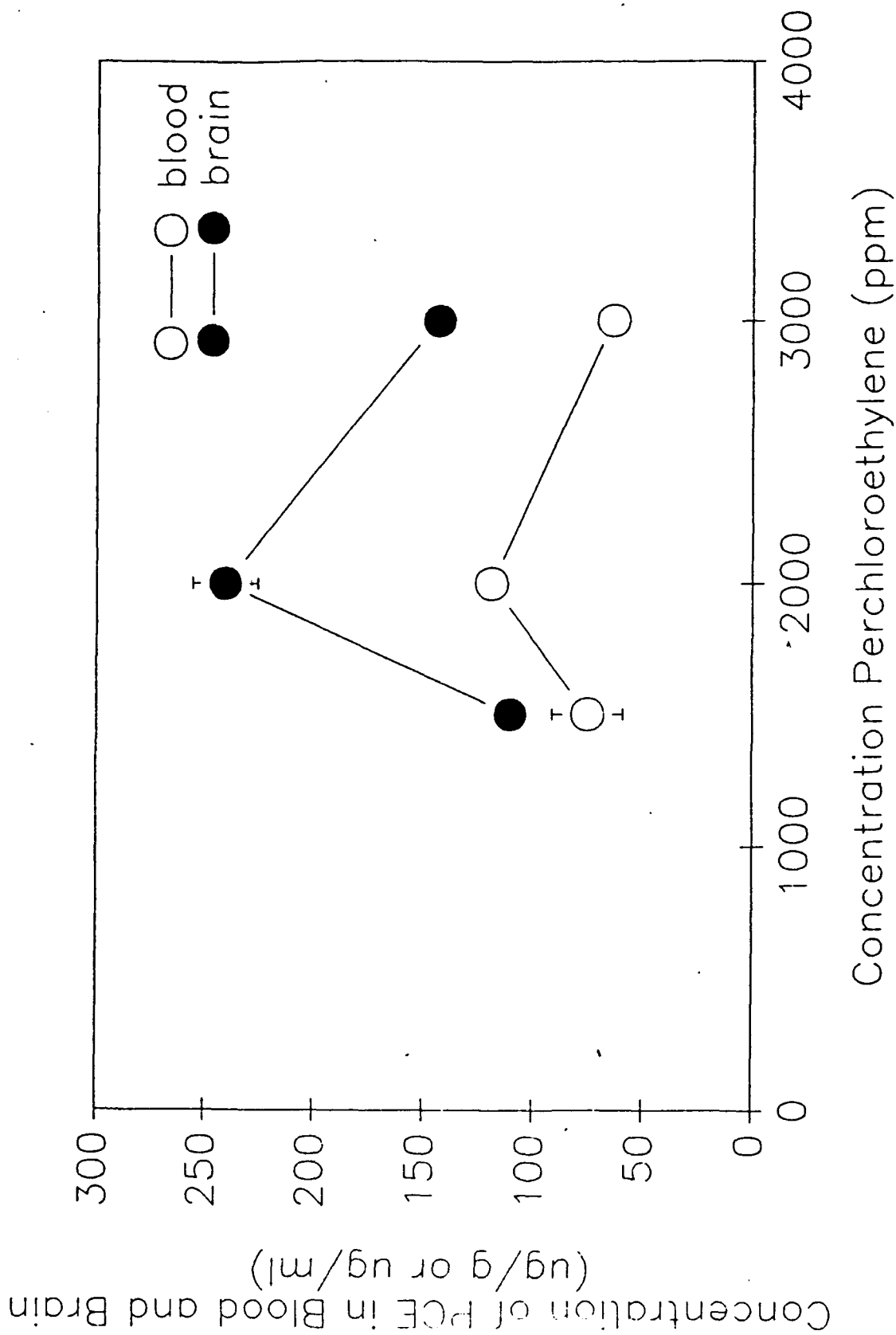


Figure 3— Blood and Brain Concentrations at Time of Fall



XI. CORRELATION OF NEUROBEHAVIORAL EFFECTS WITH TISSUE AND BLOOD PHARMACOKINETICS OF TRI IN RATS

Neurobehavioral tests are useful in assessing the acute central nervous system (CNS) effects of short-term inhalation exposures to VOCs. It is important to assess changes in CNS basal activity in a valid quantitative manner, especially when considering interspecies extrapolations. Repetitive, on-line determinations of neurobehavioral response concurrent with solvent exposure are of significant utility in elucidation of the time-course of CNS effects of the compounds. Operant performance measurements have been found to be useful for the detection of subtle CNS effects of low VOC exposure levels, prior to reaching a level which would result in irreversible neuropathological changes. CNS-depressant effects of VOCs have been demonstrated in animals by operant tests at doses similar to doses that have been shown to alter human performance.

The purpose of this study was threefold: to determine the effect of inhaled TRI on operant responding; to measure the concentration of TRI in blood and tissues during inhalation exposure; and to examine relationships between blood and brain concentrations of TRI and changes in operant behavior. Male, Sprague-Dawley rats (275-325 g) were trained to elver press for an evaporated milk reinforcer (0.08 ml) on a variable interval 30 (VI 30) schedule for 2 hr. Trained rats were exposed to clean air for 20 min, then to a single concentration of TRI (500-5000 ppm) for 100 min. The number of operant responses in each 5 min interval for the operant session was recorded. Dose-response curves were generated by plotting TRI concentrations against both mean response ratios (TRI/control) and differences in the areas under the predicted and observed cumulative response curves.

These studies of the neurobehavioral effects of PCE following inhalation exposure were presented at the 1993 meeting of the Society of Toxicology. The citation for the abstract is as follows:

Warren, D.A., Dallas, C.E., Reigle, T.G., and Christmus, W.
"Behavioral Changes During 1,1,1-trichloroethane (TRI) Inhalation in Rats: Relationship to brain and blood levels." 32nd Annual Meeting of the Society of Toxicology, New Orleans, LA; Toxicologist 13: 248, 1993.

Inhalation of 500 and 1000 ppm TRI did not decrease the rate of lever pressing, whereas 2000, 3500, and 5000 ppm TRI dose-dependently suppressed operant responding in the absence of any apparent motor impairment. Additional rats were exposed under the same conditions as those used in the behavioral study and were sacrificed at various times during exposure for blood and tissue collection. The relationships between blood and brain concentrations of TRI and changes in operant responding were examined by plotting blood and brain concentrations against mean response ratios and fitting the scatter plots with second order regression lines. Correlation coefficients for the relationships between blood and brain concentrations and operant response ratios were 0.78 and 0.80, respectively. Knowledge of how blood and brain concentrations of TRI relate to behavioral changes, coupled with a validated physiologically-based pharmacokinetic model for TRI, could be used to accurately predict TRI-induced behavioral alterations.

An operant testing system has been established in the inhalation toxicology laboratory. A modular test cage for rodents (Coulbourn Instruments) has been adapted for use in inhalation exposure chambers. Output from the cage is analyzed by a modular behavioral analysis instrument panel (Coulbourn Instruments), with component units selected for the operant tests envisioned for these studies. The performance schedules are applied with a software program (Cosmos, Coulbourn Instruments), and all data is stored on an IBM-compatible 386 computer. Different schedules and conditions are being investigated in order to optimize the results that can be obtained with each test in each species. The efficacy of the neurobehavioral testing protocols have been evaluated by the following criteria: intrasubject variability on repeated testing; intersubject variability; adaptability for use with both species; technical feasibility; rapidity with which repetitive testing can be conducted (on each subject); objectivity; sensitivity; quantitateness (i.e., ability to accurately reflect the degree of CNS dysfunction elicited over time by a range of vapor concentrations). Analysis of the data from the operant studies entailed calculating the response and reinforcement rates from the test animals during or after halocarbon exposure as a percentage of each subject's mean control values prior to exposure.

Specially tooled operant boxes were positioned inside dynamic flow inhalation chambers. Male Sprague-Dawley rats (275-325 g) were food restricted (10 g/day) and trained to elver pres for evaporated milk presentation (0.08 ml) on a VI 30 schedule for 2 hr. Once response rates were stable, rats were exposed to clean air for 20 min followed by either 500, 1000, 2000, 3500, or 5000 ppm TRI for 100 min. The number of lever presses in each 5 min interval of the operant session was computer recorded and TRI concentrations were monitored with a Miran 1B2 infrared spectrophotometer. Response ratios were calculated by dividing the number of responses during exposure by the average number of responses during the three control sessions immediately preceding exposure. Alternatively, changes in operant response rates were quantified by calculating the difference between the areas under the predicted and observed cumulative response curves during TRI exposure. Cumulative response curves were predicted by linear extrapolation of response rates during the first 20 min of each exposure session.

Male, Sprague-Dawley rats (275-325 g) were food restricted (10 g/day) and gavaged with evaporated milk (10 ml/day) to provide a diet comparable to that of the animals used in the behavioral study. Rats were exposed to 1000, 2000, 3500, or 5000 ppm TRI for either 10, 20, 40, 60, 80, or 100 min at which time they were sacrificed by cervical dislocation. Samples of blood, brain, liver, kidney, fat, muscle, heart, spleen, GI, and muscle were quickly removed and immediately placed in chilled scintillation vials containing 8 ml of isooctane and 2 ml of saline. Tissues were homogenized, vortexed, and centrifuged. Blood samples and aliquots of the tissue supernatant were placed in 20 ml headspace vials and analyzed with a headspace sampler unit of a Perkin Elmer Model 8500 gas chromatograph. The TRI concentrations in blood and tissues were calculated from a standard curve and corrected for the percent recovery characteristic of blood and tissue samples.

Operant responding was unaffected by inhalation of 500 or 1000 ppm TRI, whereas 2000, 3500, and 5000 ppm TRI decreased responding in a dose-dependent manner (Figure 1, Section H of the Appendix). The time of onset and the magnitude of the decrease were dose-dependent. The area under the curve (AUC)

method of quantifying changes in operant response rates, while resulting in a similar dose-response curve to that generated by a traditional method did not reflect the rate changes induced by inhalation of 2000 ppm TRI (Figure 2, Section H of the Appendix).

The uptake and disposition of TRI in blood and tissues were dose-dependent (Figure 3 and Figure 4). The highest concentrations of TRI were in the fat, followed by the liver and the brain. The relationships between blood and brain concentrations of TRI and mean operant response ratios were curvilinear, with correlation coefficients of 0.78 and 0.80, respectively (Figure 5). Behavioral deficits may, therefore, be reasonably predicted by blood and brain concentrations of TRI.

XII. COLLABORATIVE ARRANGEMENTS

The proposed project has been conducted at the Department of Pharmacology and Toxicology (P & Tx) and the Department of Pharmaceutics in the University of Georgia (UGA) College of Pharmacy. The Principal Investigator has been Dr. Cham E. Dallas (CED), who has been responsible for overall coordination of the project. He has provided a 20% commitment to this project. In addition to coordinating the project, CED personally conducted all of the respiratory elimination studies that were done. In that effort he developed the exposure system for the direct kinetic determinations of halocarbons, along with monitoring of respiratory parameters. Under CED's direction, the assay was developed for measuring halocarbon levels in the tissues of animals following exposure, which has been of significant utility in the present investigation. A primary focus of CED's studies has been to provide data sets for the development and validation of physiologically-based pharmacokinetic (PBPK) models. Dr. James V. Bruckner (JVB) has provided a 10% commitment to the project as a Co-Principal Investigator. He has directed a number of research projects on the oral toxicity and pharmacokinetics of volatile organic compounds over the past 10 years. JVB is also experienced in the application of pharmacokinetic data to risk assessments, having served on a number of committees and advisory groups for federal agencies concerned with health effects of VOCs. Dr. James M. Gallo (JMG) has served as a Co-Investigator (5% commitment), and provided expertise in the field of pharmacokinetics (PK). The major focus of his work has been the physiologically-based pharmacokinetic (PBPK) modeling, including the derivation of methods for estimation of mass transfer coefficients and partition coefficients for PBPK models. JMG had primary responsibility for design of PK studies and analysis of data, development and refinement of PBPK models, and assessment of the model's ability to predict halocarbon disposition in humans. Dr. Randall Tackett (RT) served as a Co-Investigator on the project (10% commitment), and was responsible for the kinetic experiments in the dog in this project. RT heads an active laboratory staffed by postdoctoral associates and graduate students, in which a number of toxicodynamic studies in the dog and the rat have been conducted. Dr. Tom Reigle (TR) also served as a Co-Investigator on the project (20% commitment). TR provided valuable assistance in the selection and purchase of the appropriate testing equipment that can be used for both rats and dogs, and was involved in the design and conduct of all the neurobehavioral studies.

Mr. Alan Warren (AW) is a doctoral student who has been conducting his dissertation research involving the research objectives of this grant. Alan is the recipient of a three-year award from the Department of Defense, managed by the Southeastern Center for Electrical Engineering Education (SCEEE). This award provides for his graduate assistantship stipend and approximately \$2000 annually for travel and minor expenses. As the SCEEE award coincides almost exactly with the period of this Air Force grant, this is an important (and much appreciated) collaborative effort. AW has personally been involved in the development of the neurobehavioral testing protocols, and has provided a very perseverant effort toward the success of this critical part of the project. Mr. Li You (LY), a doctoral student, worked on the analytical measurements of halocarbon concentrations in animal tissues, and has initiated neurobehavioral studies with mice. LY has set up the operant testing system for this second species, and has completed the initial studies for the correlation of neurobehavioral depression and halocarbon pharmacokinetics in mice.

Dr. Xiao Mei Chen (XMC) has served as a full-time postdoctoral associate on the project. XMC has a medical degree from the People's Republic of China, and has worked in the current Air Force project since its inception. She was successful in her work in the development of the assay for the measurement of halocarbons in the tissues of exposed animal and has conducted these tissue measurements thus far for ia and po exposures for PER and TET in rats and dogs. Dr. Peter Varkonyi (PV) is a postdoctoral associate from Hungary who has worked "hands-on" with the development of the PBPK models for halocarbon pharmacokinetics. Dr. Varkonyi will be replaced in the coming year by Dr. Tharin Limsakun, who has had experience with the Simusolv program we have been using to run the PBPK model simulations. Dr. Limsakin has already started on the project, and has spent the first weeks on the job learning the procedures from Dr. Varkonyi. Mr. James Durant has worked on the project as an undergraduate research assistant during the past year, with the primary responsibility for conducting the studies of neurobehavioral effects using the rotorod. Mr. Srinvasa Muralidhara has been employed part-time (25%) on the project. He participated in the analysis of halocarbons in biological samples, computer programming, the conduct of inhalation studies of dynamic exposure chambers, and the compiling of laboratory records.

Dr. Dallas and Mr. Warren went to the Medical College of Virginia for an overnight visit with Dr. Robert Balster (RB) and his laboratory in Richmond, Virginia. RB is an acknowledged leader in the study of the neurobehavioral effects of halocarbon solvents. Comparison of the theoretical and technical approaches were made in the ongoing investigation between the two laboratories. RB has also agreed to collaborate in future studies with CED's efforts in this grant, which should prove invaluable to the success of this effort.

XIII. INTERACTION WITH DOD LABORATORIES

As originally scheduled, Mr. Warren will be spending approximately 10 weeks (Oct. 1, 1993-Dec. 15, 1993) at Wright-Patterson Air Force Base (WPAFB), an opportunity afforded him as a recipient of a Department of Defense Science and Engineering Fellowship. While at WPAFB, Mr. Warren will work with his Air Force Laboratory mentor, Dr. Jeff Fisher, as well as other Air Force and contract

scientists with expertise in pharmacokinetic modeling and toxicodynamics. The studies to be conducted at this time are currently being discussed with Dr. Fisher.

APPENDIX

SECTION A

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CHROMBIO. 6660

Analyses of volatile C₂ haloethanes and haloethenes in tissues: sample preparation and extraction

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ABSTRACT

A tissue extraction procedure was developed which minimized loss of readily volatilizable compounds for subsequent quantification by headspace gas chromatography, and evaluated for perchloroethylene (PCE), 1,1-dichloroethane, 1,1,2,2-tetrachloroethane, and 1,1,2-trichloroethylene. Of the procedures evaluated, joint extraction and homogenization had the most efficient recovery, ranging from 73 to 104% for the four halocarbons from seven different rat tissues. PCE concentrations were also determined in tissues of rats following *in vivo* halocarbon administration. Recovery did not appear to be tissue-dependent, but did vary somewhat with test chemical, with the least volatile, most lipophilic compounds exhibiting the highest recovery.

INTRODUCTION

Short-chain aliphatic halogenated hydrocarbons (halocarbons) are a class of volatile organic compounds (VOCs) of increasing concern, due to their widespread occurrence as environmental contaminants and the potential risks they pose to health. Exposure to halocarbons can result in toxic injury of a number of organ systems in animals and humans. Central nervous system (CNS) dysfunction results from overexposure to most halocarbons and other VOCs [1,2]. CNS depressant effects have been directly correlated with the concentration of hydrocarbons in the brain [3,4]. Significant liver and kidney damage can be caused by certain halocarbons [5–7], while some members of this chemical class are carcinogenic

in different organ systems in animals [8–10].

Pharmacokinetic studies of halocarbons are needed in order to elucidate target organ uptake, deposition, and elimination of the chemicals. The magnitude of toxic effect in an organ is, of course, dependent upon the amount of chemical present in the tissue. Pharmacokinetic studies conducted to date have primarily involved measurement of concentrations of halocarbons in blood and exhaled breath [11–15]. The limited (*i.e.*, at a single time-point) tissue measurements conducted in some of these studies employed ¹⁴C-labeled halocarbons. Measurement of total radioactivity does not delineate between the parent compound, metabolites, and ¹⁴C which has entered the body's carbon pool. There have been a limited number of investigations, in which time-courses of tissue deposition of inhaled hydrocarbons have been delineated [3,4,16–18]. In these studies, the tissues were extracted with a solvent and the parent compounds quantified by gas chromatography (GC) or GC-mass spectral

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analysis. No reports of the time-course of uptake and elimination of halocarbons in tissues were found in the literature.

A variety of approaches have been used for analysis of VOCs in blood and tissues. One technique is to simply inject blood and tissue homogenates directly into a GC apparatus [16,19-21]. Major drawbacks of direct injection of biological materials are that the materials cause matrix interferences and that contamination markedly shortens the GC column's lifetime. Solvent extraction is a widely used approach for measuring concentrations of VOCs in blood and tissues. An aliquot of the solvent may be directly injected into the GC column [17,18,22,23]. Since this method typically involves a one-step extraction of the VOC with the solvent, limited sensitivity and interference by other lipophilic compounds can be problematic. In order to circumvent these difficulties, more complex procedures have been employed. One entails evaporation of the solvent extract and trapping of the VOC analyte on a Tenax column [24]. Another involves heating biological samples within a purging device [25-29], with subsequent retention of the analyte on an adsorbent such as Tenax. Such approaches are technically difficult and time-consuming. Headspace analysis has proven to be a sensitive and more efficient means of measuring VOC concentrations in blood samples [14,15,30,31]. No one, however, appears to have reported a suitable technique for quantification of halocarbons or other VOCs in tissues.

In light of the foregoing, it is apparent that there is a need for a rapid, sensitive analytical procedure for reliably measuring the concentrations of halocarbons and other VOCs in different tissues. The overall objective of this project was to adapt the headspace technique previously used for analysis of blood samples for measurement of C_2 halocarbons in different tissues. A major focus of the work was development of a procedure for conservation of the analyte (*i.e.*, minimization of loss by volatilization) during preparation and extraction of the tissue samples. Two C_2 haloalkanes and two C_2 haloalkenes were employed, in order to assess the utility of the proce-

dures for extraction and subsequent analyses of VOCs with different physicochemical properties.

EXPERIMENTAL

Test chemicals and apparatus

1,1,2,2-Perchloroethylene (PER), of 99% purity, and 1,1,2,2-tetrachloroethane (TET), of 97% purity, were obtained from Aldrich (Milwaukee, WI, USA). 1,1,1-Trichloroethane (TRI), of 99% purity, 1,1,2-trichloroethylene (TCE), of 99% purity, and isooctane, of 99.98% purity, were purchased from J. T. Baker (Phillipsburg, NJ, USA). A Sigma Model 300 gas chromatograph equipped with a HS-6 headspace sampler (Perkin Elmer, Norwalk, CT, USA) and a Model 5890 gas chromatograph equipped with a 19395A headspace sampler (Hewlett Packard, Avondale, PA, USA) were used for the analysis of halocarbons. Both the gas chromatographs were equipped with an electron-capture detector. Analyses were carried out on stainless-steel columns (182 cm \times 0.317 cm I.D.) packed with 10% FFAP (Alltech Assoc., Deerfield, IL, USA), 3% SP 1000 (Supelco, Bellefonte, PA, USA), or 3% OV-17 (Alltech Assoc.). Tissues were homogenized using the Ultra-Turrax SDT homogenizer (Tekmar, Cincinnati, OH, USA).

Tissue homogenization and extraction procedures

Twelve-week-old male Sprague-Dawley rats were obtained from Charles River Labs. (Raleigh, NC, USA). After two to three weeks, groups of four or eight animals (body weight = 325-375 g) were anesthetized with diethyl ether. Blood samples (1 ml) were withdrawn by closed chest cardiac puncture. Portions (0.5-1 g) of liver, kidney, brain, heart, lung, perirenal fat, and skeletal muscle were removed and placed onto ice. Each tissue was spiked with PER, TET, TCE, or TRI, by injection of 4 μ l/g tissue of a solution containing 1 mg halocarbon per ml isooctane. Two homogenization approaches were evaluated using PER. In the first, the tissues were immediately transferred after halocarbon injection to previously chilled 20-ml glass vials containing 4 ml of ice-cold saline. The tissues were allowed to

remain in the tightly capped vials for approximately 30 min. before being homogenized for an established time interval with a Tekmar tissue homogenizer. These times were kept as brief as possible in order to minimize volatilization of the halocarbons. Brain, liver, and fat were the most easily homogenized, requiring only 3–4 s. Kidney, lung and heart required 5–8 s. Skeletal muscle was the most difficult to homogenize, in that it required 20 s. Isooctane (8 ml) was added to the homogenates, which were then vortex-mixed for 30 s and centrifuged at 1800 g for 10 min at 4°C in capped vials. An aliquot of the isooctane layer was transferred to capped vials for headspace analysis. In the second approach, tissues were immediately transferred after halocarbon injection to previously chilled 20-ml glass vials containing 2 ml of ice-cold saline and 8 ml of isooctane. Each tissue was then homogenized, vortex-mixed, and centrifuged as before. An aliquot of the isooctane layer was taken for headspace analysis. Only the latter approach (*i.e.*, isooctane homogenization) was subsequently used for determination of TCE, TRI, TET, and PER in tissues, except in the aliquot volume study.

Isooctane aliquot volume study

An experiment was conducted to determine the effect of aliquot volume on the linearity of halocarbon quantification. As in other *in vitro* experiments, a Hamilton gas-tight syringe was used to inject the chemical into the center of the tissue cubes. A 4- μ l volume of PER was injected into samples of blood and each of the seven tissues. The tissues were homogenized in saline and subsequently extracted with isooctane as described previously. Aliquots (5–100 μ l) of isooctane extract were withdrawn with a pipet and transferred to 8-ml headspace vials. These vials were capped immediately with rubber septa and a spring washer and crimped to insure an airtight seal. Each sample vial was then placed into the autosampler unit of the gas chromatograph.

Headspace gas chromatographic techniques

For all the experiments with PER, the GC operating conditions were: headspace sampler tem-

perature, 90°C; injection port temperature, 200°C; column temperature, 110°C; detector temperature, 400°C; column packing, 10% FFAP; flow-rate for argon-methane carrier gas, 60 ml/min. Operating conditions for TET were: headspace sampler temperature, 100°C; injection port temperature, 200°C; column temperature, 150°C; detector temperature, 400°C; column packing, 3% OV-17; flow-rate for argon-methane carrier gas, 60 ml/min. Operating conditions for TRI and TCE were: headspace sampler temperature, 55°C; injection port temperature, 150°C; column temperature, 60°C; detector temperature, 400°C; column packing, 3% SP 1000. Except for the isooctane aliquot volume study, all analyses were conducted using a 20- μ l aliquot of isooctane in the 8-ml headspace vials. Each sample vial was heated thermostatically for 10 min in the GC autosampler unit, pressurized for 30 s with the carrier gas, and injected automatically by a solenoid-controlled mechanism into the GC column.

The aforementioned conditions resulted in vaporization of the halocarbons in the sample vials, since each VOC was heated to a temperature slightly below its boiling point, and the vial subsequently pressurized and vented into the GC. An experiment was conducted to assess the influence of heating time on quantification of PER. Heating sample vials 5 min or longer resulted in a constant GC detector response (*i.e.*, area under the curve) for a series of known quantities of PER (data not shown).

As saline was utilized in the tissue homogenization procedures, an experiment was conducted to determine whether the presence of saline influenced standard curves. PER was incorporated into four different solvent systems: 8 ml isooctane; 2 ml saline + 8 ml isooctane; 4 ml saline + 8 ml isooctane and 4 ml saline + 8 ml isooctane homogenized for 30 s. Each solution was vortex-mixed for 30 s. Aliquots of 1–25 μ l of the isooctane layer, equivalent to 1–25 ng PER, were subjected to GC headspace analysis. Standard curves were generated on the basis of the GC peak area plots. The slopes, intercepts, and correlation coefficients of the curves were compared. As described in the Results section, the standard curves

did not vary significantly from one solvent system to another. Therefore, the simplest system was subsequently employed for preparation of standard solutions of PER, TET, TCE, and TRI (*i.e.*, an appropriate amount of halocarbon was dissolved in isooctane alone). Standard curves for each compound were generated the same day that sample analyses were performed, using the same analytical conditions.

The limit of detection of the GC assay was determined by the method described by MacDougall and Chummett [32]. A signal-to-noise ratio of 3 or greater was considered as the limit of detection. The presence of background noise and any interfering peaks was assessed in air and in isooctane samples. Neither was observed, as the detector baseline was consistently stable. The limit of detection for TRI, TCE, PER, and TET was found to be 1 ng. This amount is equivalent to 8.4, 8.5, 6.7, and 6.6 parts of chemical per billion parts of air for TRI, TCE, PER, and TET, respectively.

In vivo tissue measurements

The concentration of PER was determined in tissues of rats following intra-arterial administration of the compound. Male Sprague-Dawley rats of 325–375 g from Charles River Labs. were surgically implanted with an indwelling carotid artery cannula. The cannula exited the body behind the head, so the animal could not disturb the cannula, but have freedom of movement. Food was withheld during an 18-h recovery period before PER administration. PER was incorporated into undiluted polyethylene glycol 400, and a dose of 10 mg PER/kg body weight injected as a single bolus into the arterial cannula. Each animal was anesthetized with diethyl ether 1 h after dosing, and blood taken by closed-chest cardiac puncture. Portions (1 g) of liver, kidney, brain, heart, lung, perirenal fat, and skeletal muscle were excised within 2.5 to 3 min from each animal and immediately placed into chilled vials containing 2 ml of saline and 8 ml of isooctane. The samples were processed for PER analysis using the isooctane homogenization procedure described previously.

Statistics

Comparisons of the percentage recovery of PER from tissues and blood, using the two homogenization procedures, were made using Student's *t*-test. A two-way analysis of variance was utilized to assess the significance of variances among standard curves for the different saline-isooctane mixtures. Values were considered significantly different at $p < 0.05$. The inter-assay variation had a coefficient of variation that did not exceed 12%, and the intra-assay coefficient of variation was less than 10% for all compounds tested.

RESULTS

Results of the study on the effect of isooctane aliquot volume on the linearity of halocarbon quantification are presented in Fig. 1. A very similar pattern was observed for all tissues studied. The quantity of PER increased linearly with increasing aliquot volume up to 25 μ l. Use of larger aliquots of isooctane (*i.e.*, 50 and 100 μ l) did not result in any further increase in the amount of measurable PER.

Recovery values (%) obtained using the saline and isooctane homogenization approaches for PER are contrasted in Table I. Recoveries were quite good with both procedures, in that values ranged from approximately 72 to 104%. Recoveries of PER from kidney, fat, lung, muscle, and brain were significantly higher when the tissues were homogenized directly in isooctane. Recovery of PER from liver, heart, and blood did not differ significantly for the two procedures.

Recoveries (%) of PER, TET, TCE, and TRI from spiked tissues, utilizing the isooctane homogenization procedure, are tabulated in Table II. Recovery of TET was generally higher than was the case for the other three chemicals. Recovery of TCE was generally the lowest of the four chemicals, with no mean values exceeding 88% for any tissue. Indeed, the lowest recovery of TET (*i.e.*, from fat) was greater than the highest recovery of TCE (*i.e.*, from muscle). The mean recoveries of PER, TET, and TRI from fat were quite similar (within 2%). TCE recovery (73%)

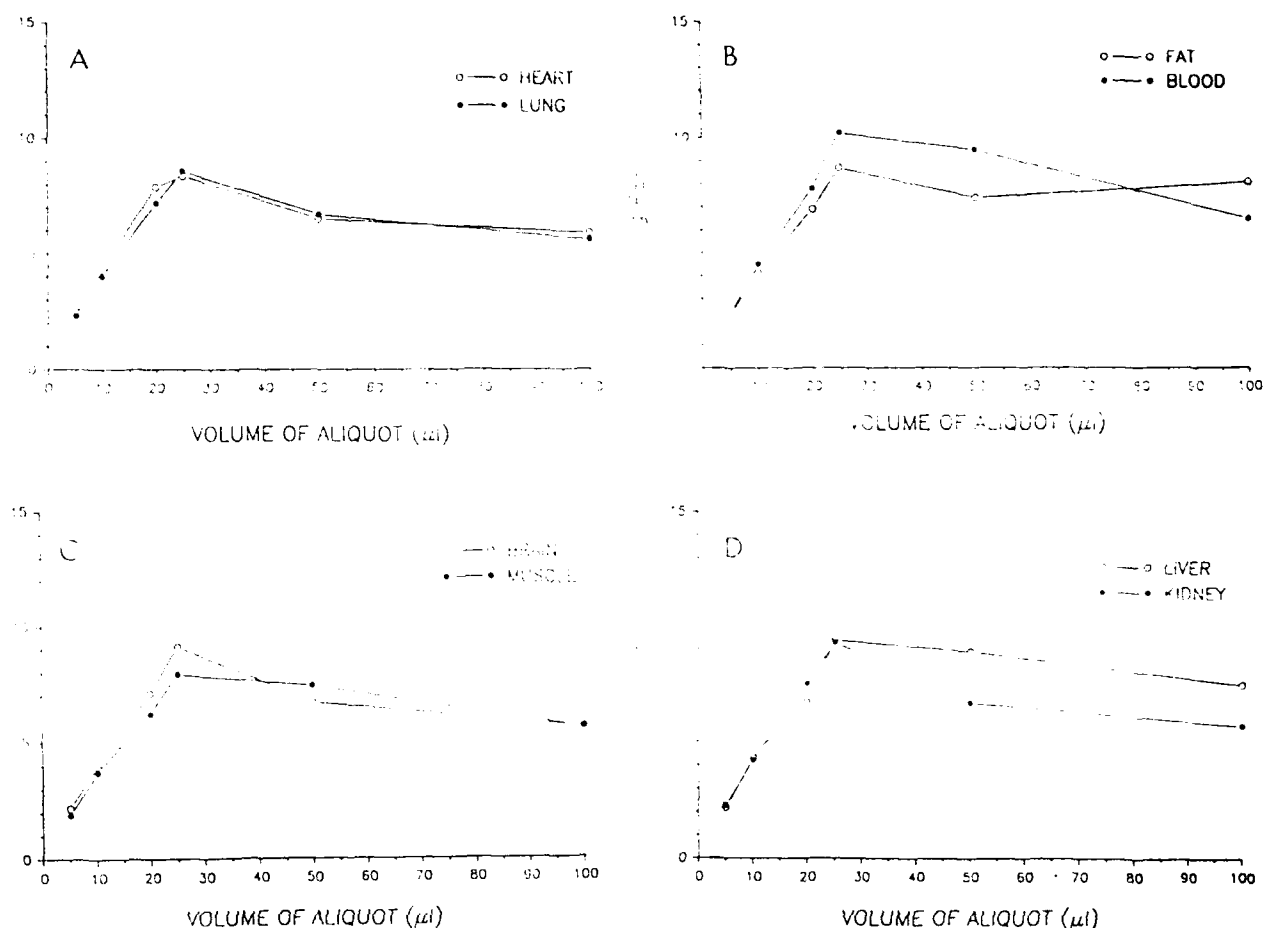


Fig. 1. Effect of isooctane aliquot volume on linearity of PER content in samples from heart and lung (A), fat and blood (B), brain and muscle (C), and liver and kidney (D). Aliquot volumes of 5, 10, 20, 25, 50, and 100 μ l were withdrawn from the organic phase of the tissue homogenate and analyzed by GC headspace analysis.

from fat was the lowest for any chemical from any tissue. The range in values for the different tissues was the smallest for TRI (*i.e.*, less than 6%) and the largest for TCE (*i.e.*, 14.9%). The recovery of the four volatile chemicals from tissues with the shortest homogenization time (*i.e.*, liver, fat, and brain) was not substantially different from that from other tissues. Unexpectedly, there was relatively high recovery of all four halocarbons from skeletal muscle, the tissue requiring the longest homogenization time. No tissue consistently exhibited higher or lower recovery values for any of the four chemicals.

Standard curves for PER standards, prepared using four different solvent and saline mixtures, are shown in Fig. 2. The linear regression equa-

tions were determined to be $y = 25.9x - 13.3$ for 8 ml isooctane, $y = 25.9x - 8.4$ for the 2 ml saline–8 ml isooctane mixture, $y = 25.8x - 8.0$ for the 4 ml saline–8 ml nonhomogenized isooctane mixture, and $y = 25.2x - 3.6$ for the 4 ml saline–8 ml homogenized isooctane mixture. For all of the data considered together, the linear regression equation was $y = 24.3x - 6.9$. Thus, there was no statistically significant difference between the regression equations for the four solvent systems. Therefore, neither the presence of saline nor homogenization significantly affected standard curves for PER.

Concentrations of PER, measured in tissues of rats 1 h following intra-arterial administration of a single 10 mg/kg dose of PER, are shown in Ta-

TABLE I
EFFECT OF HOMOGENIZATION PROCEDURE ON THE RECOVERY OF PER

Each value represents the mean \pm S.E. for recovery of 1,1,2,2-tetrachloroethylene (PER) from tissues of eight rats for isooctane homogenization and four rats for saline homogenization.

Sample	Recovery (%)	
	Saline homogenization	Isooctane homogenization
Liver	95.5 \pm 9.9	89.6 \pm 3.1
Kidney	69.0 \pm 4.9 ^a	86.7 \pm 1.4
Fat	73.8 \pm 4.9 ^a	88.2 \pm 2.7
Heart	75.8 \pm 10.3	81.2 \pm 1.2
Lung	78.8 \pm 7.1 ^a	99.1 \pm 2.3 ^b
Muscle	80.3 \pm 6.3	98.5 \pm 2.9
Brain	72.3 \pm 8.7 ^a	88.6 \pm 2.0
Blood	104.8 \pm 10.1 ^a	95.4 \pm 4.1

^a Significant difference between procedures at $p < 0.05$.

^b Significant difference between procedures at $p < 0.01$.

^c Significant difference between procedures at $p < 0.001$.

ble III. PER levels in the fat were an order of magnitude or more higher than in any other tissue sampled. The concentrations of PER measured in the liver, kidney, heart, and lung were relatively consistent (*i.e.*, within 25% of each other). Brain levels of PER were approximately two-

fold higher than in these organs, while the blood and skeletal muscle exhibited the lowest concentrations.

DISCUSSION

GC techniques are routinely used to determine levels of VOCs in environmental and biological samples. Wallace *et al.* [33] utilized GC purge-and-trap techniques to conduct large-scale surveys of human exposure to VOCs in drinking water, indoor and outdoor air. GC purge-and-trap techniques have also been used successfully to measure concentrations of VOCs in human blood [27,29,30,34], milk [26,27], and urine [27]. These assays are precise and quite sensitive, as shown by the investigators' employed GC/mass spectrometric computer analyses. Other investigators have used static GC headspace methods to quantify halocarbons and other VOCs in blood [14,15,30,31]. Such headspace analyses offer the advantages of speed and simplicity, such that large numbers of samples can be assayed daily using a gas chromatograph equipped with an autosampler. Although each of the aforementioned techniques generally work well for air and liquids, little attention has been devoted to adapting them for measurement of VOCs in solid tissues.

TABLE II
RECOVERY OF C₂ HALOALKANES AND HALOALKENES FROM BLOOD AND TISSUES

Values represent the mean \pm S.E. for measurement in spiked tissues taken from eight rats. Each spiked tissue was homogenized in 8 ml ice-cold isooctane and 2 ml saline, vortex-mixed, centrifuged at 4°C, and an aliquot of the isooctane assayed by headspace GC as described in Experimental.

Sample	Recovery (%)			
	PER	TET	TCE	TRI
Liver	89.6 \pm 3.1	96.1 \pm 2.1	86.0 \pm 1.3	91.3 \pm 4.0
Kidney	86.7 \pm 1.4	97.9 \pm 1.5	86.9 \pm 1.6	88.4 \pm 4.9
Fat	88.2 \pm 2.7	89.9 \pm 1.4	73.0 \pm 1.5	88.7 \pm 2.2
Heart	81.2 \pm 1.2	98.1 \pm 1.4	85.9 \pm 3.5	89.6 \pm 2.4
Lung	99.1 \pm 2.3	96.1 \pm 0.8	80.0 \pm 0.7	89.7 \pm 1.9
Muscle	98.5 \pm 2.9	97.4 \pm 1.1	87.9 \pm 1.7	87.6 \pm 4.9
Brain	88.6 \pm 2.0	100.3 \pm 3.1	80.7 \pm 2.8	87.6 \pm 4.6
Blood	95.4 \pm 4.1	97.3 \pm 2.1	85.9 \pm 2.0	85.5 \pm 3.7

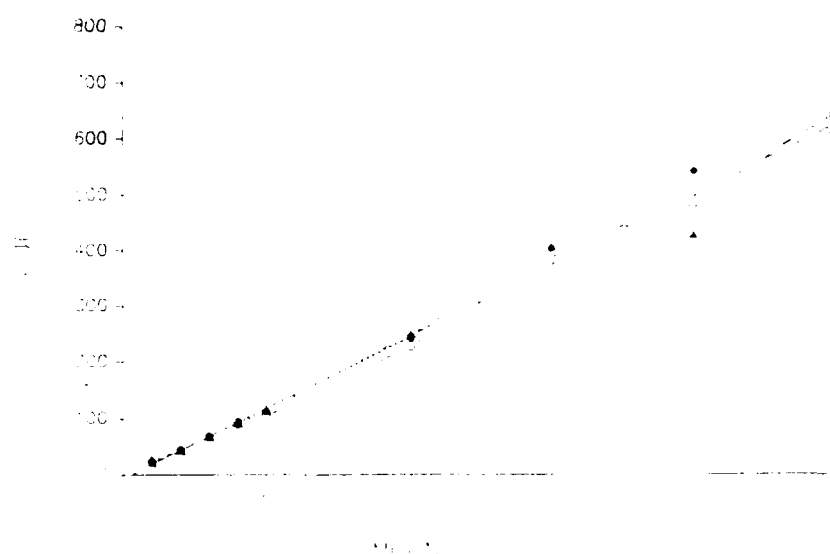


Fig. 2. Standard curves for PIR using four solvents: isooctane (●), n-hexane (▲), n-pentane (■), and n-butane (○). The amount of solvent used was 0.5 ml. Isooctane (●), n-hexane (▲), n-pentane (■), and n-butane (○) were analyzed for 80 s. The detector response, presented here as area under the curve (AUC), is plotted against amount of PIR. The regression equation for the data presented here is $y = 24.3x + 6.9$.

A practical technique for processing and extracting C_2 halocarbons from tissues for subsequent GC headspace analyses is reported here. Several approaches for determination of VOCs in tissues have been employed previously, with limited success. Direct injections of tissue homog-

enates or solvent extracts or homogenates have a number of inherent problems, including loss of the VOC by volatilization, GC column contamination, interference by biological matrices and lipophilic macromolecules, and limited sensitivity. One method for measuring toluene in blood and tissues involved extraction with methanol, selective adsorption onto Tenax, and desorption from the Tenax with heat into a gas chromatograph [24]. A significant problem in measuring VOCs in solid tissues is volatilization of the analyte during tissue processing. Peterson and Bruckner [24] attempted to overcome this difficulty by crushing the tissues with a rod under methanol within a closed container. This technique was reasonably successful (*e.g.*, 73 and 92% recovery of toluene from liver and brain, respectively), but recoveries from other tissues were limited by incomplete maceration and escape of toluene from the maceration-extraction container. This technique was also labor-intensive and time-consuming, as was a purge- and-trap method described by Lin *et al.* [28] for measuring 1,1- and 1,2-dichloroethylene (1,2-DCE). The procedure of Lin *et al.* [28] involved thermal desorption of halocarbons from previously

TABLE III

TISSUE CONCENTRATIONS OF PER IN RATS FOLLOWING *IN VIVO* EXPOSURE

Each animal was sacrificed 1 h after intra-arterial administration of 10 mg 1,1,2,2-tetrachloroethylene (PER) per kg body weight.

Tissue	PER concentration ^a ($\mu\text{g/g}$)
Liver	2.4 ± 0.5
Kidney	2.7 ± 0.4
Fat	48.1 ± 4.1
Heart	3.0 ± 0.2
Lung	2.3 ± 0.2
Muscle	1.9 ± 0.2
Brain	4.7 ± 0.4
Blood	1.3 ± 0.1

^a Values represent the mean \pm S.E. for four rats.

minced tissues within a purging device. The chemicals were subsequently trapped on a Tenax column and desorbed with heat into a gas chromatograph. Mean recovery values for 1,2-DCE from liver, kidney, brain, and adipose tissue were 60, 53, 63 and 93%, respectively [28]. The purge-and-trap technique of Pellizzari and co-workers [27,35] also resulted in low recovery (*i.e.*, 13–80%) of a series of halocarbons from adipose tissue. The method involved transfer of 5-g portions of frozen adipose tissue to a 100-ml round-bottom purging flask maintained in an ice bath, addition of the halocarbon dissolved in distilled water, and maceration with a Virtis tissue homogenizer. The flask's contents were then heated to 50 °C, stirred and purged with helium for 30 min, in order to transfer the analyte to a Tenax column. These researchers [27,35] attributed their low recovery values and marked inter-sample variability to halocarbon losses during tissue maceration and transfer, as well as retention of the analyte by complex matrices and lipophilic compounds. In contrast, the technique presented in the current paper is quite efficient, in that it involves a homogenization-extraction step and the relative ease and speed of GC headspace analysis. The method was also sensitive (*i.e.*, limit of detection = 1 ng) and efficient, in that recoveries of four different halocarbons from a variety of tissues were quite high (*i.e.*, 73–104%) and consistent (*i.e.*, highest S.E. = 4.9%).

An important factor in the present procedure was the limitation in the volume of aliquot that could be employed in the headspace vials. As standard curve measurements were no longer linear at volumes above 25 μ l, a 20- μ l aliquot was selected for subsequent use. There was a statistically higher recovery from most tissues when using isooctane homogenization than when using saline homogenization. It appears that homogenization of tissues in an aqueous solution (*i.e.*, saline), with subsequent extraction into isooctane, provided more opportunity for loss of the volatile chemicals through evaporation than did the single step of homogenization in isooctane. Isooctane proved to be superior to a variety of other organic solvents for extraction of all four

halocarbons (unpublished data). Other solvents that were employed included methanol, ethyl acetate, *n*-hexane, cyclohexane, and toluene. Some solvents (*e.g.*, ethyl acetate) worked well for one halocarbon, but not for others. Isooctane provided the highest recovery without interfering peaks for all four halocarbons.

The applicability of this approach for analysis of C₂ halocarbons was demonstrated by its use with two haloalkanes and two haloalkenes with differing physicochemical properties. As would be expected, the relative volatility of the chemicals affected the recovery. The boiling points of TRI, ICE, PER, and TET are 74, 86.7, 121, and 140.7 °C, respectively [36]. TET, the least volatile chemical, exhibited the highest recovery from most tissues. Recovery of PER was also relatively high from each tissue except the heart. It is noteworthy that PER is the most lipophilic of the halocarbons [37], and therefore should be most efficiently extracted by isooctane. ICE generally exhibited the lowest recovery values, as would be anticipated from its relatively high volatility and low lipophilicity. Recovery of TRI was unexpectedly high from most tissues. One would predict that TRI recovery should also be relatively low, since it was the most volatile and one of the least lipophilic of the four compounds studied.

A basic tenet of toxicology is that of the dose-response relationship (*i.e.*, the magnitude of toxic effect is a function of the administered dose). The concept of dose is now being refined, as it is recognized that the amount of chemical absorbed systemically (*i.e.*, the internal dose) can vary significantly with route of exposure, dosing vehicle and animal species. The blood level over time following exposure has been accepted historically as an index of internal dose, but it often may not accurately reflect concentration of chemicals at sites of action within tissues. Thus, the most logical and precise measures of dose are time integrals of target organ concentrations of bioactive chemicals [38]. Unfortunately, there are a paucity of tissue level *versus* time data sets for VOCs, due largely to the technical difficulties and the inordinate time involved in quantification of these highly volatile compounds in individual samples.

In order to derive appropriate time-course data, tissue concentrations must be measured sequentially during and post exposure in separate groups of animals, necessitating analysis of a large number of samples. A technique is presented here, which allows rapid processing and extraction of C₂ halocarbons from a variety of organs, for subsequent GC headspace analyses. By use of such a procedure, it should be possible to generate comprehensive tissue dose-time-course data to correlate with toxicity data. Recognition and utilization of such information can substantially reduce uncertainties inherent in toxicity and carcinogenicity risk assessments of halocarbons and other VOCs.

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APPENDIX

SECTION B

Manuscript now being submitted to
Toxicology and Applied Pharmacology

Development of a Physiologically Based Model for Perchloroethylene
Pharmacokinetics Using Tissue-Concentration Time Data

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ABSTRACT

Development of a Physiologically Based Model for Perchloroethylene Pharmacokinetics Using Tissue-Concentration Time Data. DALLAS, C.E., CHEN, X.M., O'BARR, K., MURALIDHARA, S., VARKONYI, P., AND BRUCKNER, J.V. (1993) Toxicol. Appl. Pharmacol. 0, 00-00.

The tissue disposition of perchloroethylene (PCE) was determined experimentally in rats in order to 1) obtain model input parameters from in vivo data for the development of a physiologically based pharmacokinetic (PBPK) model, and 2) forecast the disposition of PCE in a variety of tissues following inhalation exposure. For the derivation of model input parameters, male Sprague-Dawley rats received a single bolus of 10 mg PCE/kg bw in polyethylene glycol 400 by intraarterial injection through an indwelling carotid arterial cannula. For the testing of the PBPK model simulations, male Sprague-Dawley rats inhaled 500 ppm PCE for 2 hours in dynamic exposure inhalation chambers. Serial samples of brain, liver, kidney, lung, heart, skeletal muscle, perirenal adipose tissue, and blood were taken for up to 48 hr following ia administration and during inhalation exposure and up to 72 hr post-exposure. Blood and tissue PCE concentrations were analyzed using a gas chromatography headspace technique. Following ia administration, there were similar terminal elimination half-lives ($t_{1/2}$) for each of the tissues. As comparable tissue $t_{1/2}$ were consistent with a blood-flow limited model, tissue-blood partition coefficients were calculated for non-eliminating compartments by division of the tissue area-under-the-tissue-concentration-time curve (AUC) by the blood AUC. For the liver, the first-order metabolic rate constant and tissue PCE concentration were also employed in the calculation. A PBPK model was developed using these parameters derived from ia data and used to make predictions of the tissue concentrations in each of the

model compartments during and following PCE inhalation exposure. Overall, predicted tissue concentrations were in agreement with the direct measurements conducted over time in the seven tissues and in blood. Tissue concentration time data can thus provide valuable input for halocarbon PBPK model parameter estimates, which resulted in a PBPK model in which there is greater confidence in the accuracy of the tissue compartments which comprise the model.

INTRODUCTION

A fundamental basis in the development of physiologically based pharmacokinetic (PBPK) models in the division of the body into anatomical compartments representing individual organs or groups of organs which share a common characteristic. A mass balance differential equation is written for each compartment in the model, based upon anatomical and physiological parameters (i.e., tissue volumes and blood flow rates) for the test species and upon physicochemical parameters (i.e., partition coefficients and biochemical constants) for each chemical (Gerlowski and Jain, 1983). Solution of the set of mass balance differential equations generates chemical concentrations as a function of time in each compartment/tissue. Obviously, evaluation of the accuracy of the predicted chemical concentrations in each compartment is important to determining model accuracy since the model to a significant extent is comprised of these tissue compartments.

Although knowledge of the deposition of chemicals in the tissues is of major importance in model evaluation, most testing of the accuracy of PBPK model predictions concerning the uptake and elimination of volatile organic compounds (VOCs) has been from comparison to observed values in the blood or exhaled breath. Blood level over time, as a measure of bioavailability, has classically been accepted as an index of the level of chemical in the body, and therefore a representative index of toxic effects. This assumption can be misleading, in that blood concentrations may not be reflective of the concentration of chemical or active metabolite at the site of action in a target tissue. Thus, a more logical measure of target organ exposure is the area under the tissue concentration versus time curve for the reactive chemical (Andersen, 1987a).

Unfortunately, only a limited number of published studies have tested the ability of PBPK models to accurately predict time integrals of tissue exposure to VOCs, apparently due to the paucity of tissue concentration versus time data sets. This lack of data is due to the considerable effort required in such studies and to technical problems with quantitation of the volatile chemicals in solid tissues. In a typical case, the only tissue data Reitz et al. (1988) had to use for testing their TRI model were levels of radioactivity measured by Schumann et al. (1982) in the liver and fat of mice and rats at the termination of 6-hour inhalation exposures to ^{14}C -TRI. Measurement of radioactivity, of course, does not distinguish between parent compound, metabolites and ^{14}C which has entered the carbon pool. It should also be recognized that VOCs are very rapidly absorbed from the lung (Dallas et al., 1983; Dallas et al., 1989) and GI tract (D'Souza et al., 1985; Putcha et al., 1986) into the bloodstream, transported to tissues, and quickly eliminated post exposure. Manifestation of CNS effects (Bruckner and Peterson, 1981) and cytotoxicity (Rao and Recknagel, 1968; Lowry et al., 1981; Luthra et al., 1984) occur within minutes of the onset of VOC exposure. Thus, it is important to delineate time-courses of tissue uptake and elimination of selected VOCs, particularly during the critical "early period" of exposure when many important events in cellular dysfunction and injury occur.

Toward this end, tissue-concentration time data sets were generated for perchloroethylene (PCE) exposures in rats. Intraarterial administration of this VOC was utilized to generate data to be utilized in the in vivo derivation of critical PBPK model parameters, particularly tissue:blood partition coefficients. A PBPK model developed on this basis was then tested for model accuracy in its tissue compartments by comparison to observed tissue concentration-time data

during and following inhalation exposure to PCE. In this way, useful model parameters and evaluation of the validity of model compartments were procured from detailed measurements of tissue uptake and elimination.

METHODS

Male beagle dogs (6-15 kg) obtained from Marshall Farms (North Rose, NY), adult male Sprague-Dawley rats (325-375 g), obtained from Charles River Laboratories (Raleigh, NC), were employed in these studies. The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. Rats were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow® were provided ad libitum. The rats were used after at least a 7-day acclimation period, at which time they were approximately 12 weeks old. Solvent exposures were initiated between 1000 and 1200 hr each day.

1,1,2,2-Perchloroethylene (PCE) (tetrachloroethylene) of 99%+ purity was obtained from Aldrick Chemical Co. (Milwaukee, WI). The purity of the chemical during the conduct of the study was verified by gas chromatography. For intraarterial (ia) administrations to rats, a cannula was surgically implanted into the common carotid artery. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml):acepromazine maleate (10 mg/ml):xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v/v/v). The cannulated animals were allowed relative freedom of movement during a 24-hr recovery period with the cannulas protected from manipulation by surgical tape at the back of the head. All animals employed in the tissue kinetic studies were unanesthetized. For ia administration, PCE

was given as a single bolus at a dose of 10 mg/kg, using polyethylene glycol 400 volume/kg bw as a vehicle.

Inhalation exposures of freely moving animals were conducted in 1.0 M³ Rochester-type dynamic flow chambers. Test atmospheres of halocarbons were generated by vaporization of the compounds under different combinations of air flow- and temperature-controlled schedules. A constant flow of liquid chemical was infused into a glass dispersion flask using a synchronous drive flow pump (Fluid Metering, Inc.). Air or nitrogen was passed through the dispersion flask at a constant rate and directed into the chamber influent air stream. A heating mantle was placed around the dispersion flask, and narrowly controlled temperature limits maintained for volatilization continuity. The entire halocarbon-generating system was enclosed in specially fabricated safety boxes. The boxes were maintained under constant negative pressure during inhalation exposures in the same manner as the inhalation chambers. Exhaust air from the box chambers and the generation box were vented to HEPA and charcoal filters, so that all air with any chemical was scrubbed before release to the environment.

The chambers were operated at flow rates of 7 to 15 ft³ per minute (1/4 to 1/2 changes of the chamber volume per minute). A negative pressure between 20 and 50 mm was maintained at all times during operation of the chambers. Concentrations of the halocarbon vapors in the chamber air were monitored by withdrawing one ml samples of air from the chamber and directly injecting the samples into a Tracor MT560 gas chromatograph (Tracor Instruments, Austin, TX). Standards were prepared in each of four 9-liter bottles equipped with Teflon[®] stoppers with needles from which air samples could be taken by syringe. Analyses for monitoring the 500 ppm exposures were conducted using a flame ionization detector (FID). Air samples were procured with a gas-tight, 1-ml syringe and

injected directly onto an 8-ft X 1/8-in stainless steel column packed with 0.1% AT 1000 on GraphPak. Operating temperatures were: 150°C, injection port; 200°C, FID detector; 350°C, ECD detector; 110°C, isothermal column operation.

In a typical exposure sequence, groups of 5 rats were placed into each of a set of wire-mesh exposure cages and positioned in the exposure chamber. Each animal was individually housed in the cage, so that the animals cannot limit its inhalation of the halocarbon vapors by burying its nose in one another's fur.

Groups of animals (n=4 rats) were serially sacrificed (using etherization) at the following times following a dosing: 1, 5, 10, 15, 30, and 60 min, and 1, 2, 4, 6, 12, 36, 48, and 72 hr. For inhalation exposures, sampling times were at 15, 30, 60, 90, and 120 minutes during exposure, and 0.25, 0.5, 1, 2, 4, 6, 12, 24, 36, 48, and 72 hrs post-exposure. Blood samples were obtained by cardiac puncture. Approximately 1 g samples of liver, kidney, brain, lung, heart, perirenal fat, and skeletal muscle were then quickly removed and placed into previously chilled 20-ml glass vials containing 2 ml of ice-cold saline and 8 ml of isooctane. Each tissue was then homogenized for an established time interval with a Ultra-Turrax SDT homogenizer (Tekmar Co., Cincinnati, OH). These times were kept as brief as possible in order to minimize volatilization of the halocarbons. Brain, liver, and fat were the most easily homogenized, requiring only 3-4 sec. Kidney, lung and heart required 5-8 sec. Skeletal muscle was the most difficult to homogenize, in that it required 20 sec. The homogenates were then centrifuged at 1800 x g for 10 min at 4°C in capped vials. An aliquot of the isooctane layer was transferred to 8-ml headspace vials. These vials were capped immediately with rubber septa and a spring washer and crimped to insure an airtight seal. Each sample vial was then placed into the autosampler unit of a gas chromatograph (GC). The limit of detection for PCE was approximately 1 ng,

or 6.7 parts of PCE per billion parts of air in the sample vial. Percent recovery of the procedure for PCE for each tissue was employed in determining the final tissue concentration, as described by Chen et al. (1992).

A model Sigma 300 GC equipped with a HS-6 headspace sampler (Perkin Elmer Co., Norwalk, CT) was used for the analysis of the halocarbon. The GC was equipped with an electron capture detector. Analyses were carried out on stainless-steel columns (182 cm x 0.317 cm) packed with 10% FFAP (Alltech Associates, Deerfield, IL). The GC operating conditions were: headspace sampler temperature, 90°C; injection port temperature, 200°C; column temperature, 110°C; detector temperature, 400°C; flow rate for argon/methane carrier gas, 60 ml/min. All analyses were conducted using a 20- μ l aliquot of isooctane in the 8-ml headspace vials. Each sample vial was heated thermostatically for 10 min in the GC autosampler unit, pressurized for 30 sec with the carrier gas, and injected automatically by a solenoid-controlled mechanism into the GC column. The aforementioned conditions resulted in vaporization of the halocarbons in the sample vials, since PCE was heated to a temperature slightly below its boiling point, and the vial subsequently pressurized and vented into the GC.

The disposition of PCE during and following inhalation exposures in the rat was predicted using a physiologically-based pharmacokinetic (PBPK) model for po (Fig. 1a) and ia (Fig. 1b) administration. It was similar to PBPK models previously developed by Angelo and Pritchard (1984) and Ramsey and Andersen (1984) for other VOCs, in that it provided for inhalation and exhalation through a lung compartment, metabolism in a liver compartment, and blood-flow-limited organ representations. The current model differed primarily in that it included a greater number of tissue compartments. Values determined in this laboratory were employed for tissue volumes and blood flows (Delp et al., 1991) and alveolar

ventilation (Dallas et al., 1991) for the male Sprague-Dawley rat. In vivo tissue:blood partition coefficients were calculated from ia data obtained in the present study by the area method of Gallo et al., (1987). The metabolic parameters K_m and V_{max} , and blood:air coefficients were estimated from the observed data by nonlinear regression analysis.

The area under the concentration-time curve (AUC) for blood and tissues was determined from the time of administration to infinity. Total body clearance was determined as the dose divided by the blood AUC in each species. Bioavailability was calculated by AUC_{po}/AUC_{ia} . The maximum concentration reached in blood and tissues (C_{max}) and the time after dosing that it occurred (T_{max}) were determined by observation of the available data points.

Differential mass balance equations, incorporating the parameters listed in Table 1, that described the transport of PCE in the rat were numerically integrated with the Advanced Continuous Simulation Language (ACSL) computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted PCE concentrations over time. The model-predicted cumulative uptake values were equal to the sum of the simulated amounts of PCE in each tissue compartment in the model.

RESULTS

In the measurement of PCE in tissues following ia administration (Figs. 2 and 3), PCE appeared to be eliminated somewhat more rapidly from the liver than from other tissues. All of the extrahepatic tissues exhibited quite similar $t_{1/2}$ values, ranging from 389-496 min after ia injection (Table 1). As would be anticipated for a chemical as highly lipophilic as PCE, the C_{max} and AUC values

for the adipose tissue were substantially greater than for all other tissues following ia administration.

The degree of blood perfusion had a significant impact on tissue disposition of PCE in the rat. Highly perfused organs such as kidney and brain had similar C_{max} , AUC, and $t_{1/2}$ values for each route of administration. Poorly perfused and nonlipoidal tissues, such as skeletal muscle, had lower C_{max} and AUC values than these highly perfused tissues.

Tissue:blood partition coefficients were calculated from the ia tissue AUC data, and are presented in Table 2. Due to the high degree of lipophilicity of PCE, the fat:blood value was almost two orders of magnitude greater than for the nonfat tissues. The highly perfused tissues such as liver, kidney, and brain had similar values, and were higher than the other nonfat tissue values.

Pharmacokinetic parameter estimates following the 2 hr inhalation exposure to 500 ppm PCE are presented in Table 3. The C_{max} achieved in fat tissues was 9-18 times higher than in the nonfat tissues. The tissue AUC for fat was also 45-80 times higher than AUC values for the nonfat tissues. Among nonfat tissues, the brain had the highest values for AUC, $t_{1/2}$, and C_{max} . These values were similar to those observed for the liver during inhalation exposure. As a generally poorly perfused tissue, the muscle had the lowest C_{max} and a relatively low $t_{1/2}$.

Using the parameter estimates from the ia tissue disposition data, a PBPK model was developed for predicting the pharmacokinetics of PCE in tissues during and following inhalation exposure. Predictions of PCE tissue concentrations are compared to the experimentally determined (i.e., observed) concentration versus time profiles (Figs. 4 and 5). Overall, concentrations of PCE both during and following inhalation exposure were well predicted by the model. Predictions of

PCE concentrations in the liver and kidney were in close agreement during and immediately following exposure, with slight overpredictions during the terminal elimination phase (Figs. 4a & b). Concentrations of PCE in the fat were accurately predicted during inhalation, with a slight but consistent underprediction during inhalation for heart, lung, brain, and blood (muscle concentrations were well predicted during exposure), and for all four tissue groups and the blood immediately following exposure. During the terminal elimination phase, predictions of PCE in all five groups was slightly overpredicted.

DISCUSSION

PBPK model-predicted PCE concentrations in rat tissues in the present investigation were in close agreement with direct measurements of the chemical over time. Previous validations of PBPK models for PCE and most other volatile organic halocarbons (VOCs) have primarily relied on blood and exhaled breath data (Chen and Blancato, 1987; Travis, 1987; Ward et al., 1988). For example, the model of Reitz et al. (1988) reliably forecast blood and exhaled breath levels of TRI in mice, rats and humans during and following inhalation exposures. Their PBPK model was also versatile enough to forecast the kinetics of TRI in rats given the chemical iv and orally, as well as predicting target organ (i.e., liver) concentrations in humans who may consume trace levels of TRI in their drinking water. However, no tissue data were available for humans or other species for verification of the model predictions of target organ concentrations.

There have been relatively few attempts to model the uptake and elimination of halocarbons and other VOCs in tissues. In papers published to date (Ramsey and Andersen, 1984; Reitz et al., 1988; Travis et al., 1990), the authors had to

utilize the limited available tissue-concentration time-course data of other investigators. In the current investigation, a PBPK model was developed to describe the time-course of blood and tissue concentrations of PCE, utilizing tissue concentration-time data following intraarterial injection into rats. This model differs from most previously published PBPK models for VOCs primarily in that it includes a greater number of tissue compartments. Additionally, most of the physiological input parameters (i.e., respiratory minute volume, cardiac blood flow, individual tissue blood flows, and tissue volumes) were carefully measured in rats of the same age/body weight as those used in the VOC exposures (Dallas et al., 1991; Delp et al., 1991; Manning et al., 1992). Partition coefficients were calculated by the "area" method of Gallo et al. (1987), which takes into account specific organ structure, blood flow limitation and observed tissue concentration versus time data. The blood:air partition coefficient that was determined for PCE, 18.9, was quite similar to an *in vitro* value (19.6) published by Gargas et al. (1989). The tissue:blood partition coefficients in the current *in vivo* study were 1.4, 1.8, and 2.8 times higher than the liver, fat, and muscle values, respectively, reported using the vial equilibration technique (Gargas et al., 1986 & 1989). These *in vitro* partition coefficients proved to be significantly useful in predicting PCE expiration in rats (Travis, 1987; Ward et al., 1988) relative to observed data (Pegg et al., 1979; Buben and O'Flaherty, 1985). However, it was necessary to modify the fat tissue partition coefficient to adjust model fits to experimental data. In the current study, predicted tissue concentration versus time data agreed quite well with VOC levels measured in tissues during and following PCE inhalation exposures (i.e., liver, kidney, brain, heart, blood, lung, skeletal muscle and fat). Thus, the new model

is a more detailed and accurate description of physiological structure, and reliably forecasts blood and tissue time-courses in rats.

It has frequently been necessary during the development of the PBPK models for VOCs to manipulate anatomical, physiological and physicochemical input parameters, in order to obtain adequate simulations of observed/experimental data. Ramsey and Andersen (1984), for example, had to make the following changes in their PBPK model for styrene to obtain good agreement between observed and predicted values: increase in the fat tissue volume and blood perfusion rate; decrease of the fat:blood partition coefficient; increase of the blood flow rate to the metabolizing tissue group; and make offsetting alterations in the lean muscle and richly perfused tissue groups. Dallas et al. (1989) developed a PBPK model which accurately predicted blood and exhaled breath levels of TRI, which underpredicted cumulative (i.e. total) uptake of TRI during inhalation exposures. In this study, it was assumed that the tissue:blood partition coefficient for the liver and for richly perfused tissues was to be 1.49 (as cited by Gargas et al., 1989). However, if blood and liver concentrations of TRI measured in rats at the end of a 6-hour exposure (Schumann et al., 1982; Reitz et al., 1988) were used to calculate *in vivo* liver:blood coefficient, a value of 3.45 would be obtained. Use of this higher value for the liver and richly perfused tissues would result in an increase in predicted uptake (i.e. absorbed dose), as would inclusion of additional tissues (e.g. brain, pancreas, adrenals, testes) with a high affinity for TRI in the richly perfused compartment. Indeed, using the tissue:blood partition coefficients from the *ia* tissue concentration time data in this investigation cumulative uptake of PCE in a companion study (Dallas et al., 1993) was well-predicted during inhalation exposures, as were blood and exhaled breath concentrations.

The use of tissue concentration time data can therefore be of significant utility in the development of PBPK models. Model parameter estimation is aided for tissue:blood partition coefficients, as direct measurements can be used to determine these values for as many model compartments as is feasible in the experimental design. Determination of the reliability of the model predictions of tissue concentrations in each compartment using this approach could be useful in improving the accuracy of risk assessments related to target organ disposition of VOCs.

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Table 1
Pharmacokinetic Parameters in the Rat Following
Intraarterial Administration of 10 mg PCE/kg bw¹

Tissue	Area under curve ($\mu\text{g}\cdot\text{min}/\text{ml}$)	Biological half-life (min)	C _{max} ($\mu\text{g}/\text{g}$)	T _{max} (min)
Liver	1737	389	41.0	1
Kidney	1759	412	36.0	1
Fat	60335	466	64.4	30
Heart	1059	439	19.0	1
Lung	909	479	11.3	1
Muscle	1178	443	5.7	10
Brain	1730	443	21.7	1
Blood	396	496	4.6	1

¹Each value represents the mean for 4 rats and 14 time points ranging from 1 min to 72 hr.

Table 2
Parameters Used in the Physiologically Based
Pharmacokinetic Model for PCE in the Rat

Parameter	
Alveolar ventilation (ml/min)	108
Inhaled Gas Concentration (mg/ml)	3.55 (500 ppm)
Body Weight (kg)	340
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Tissue volumes (ml)	Percentage of Body Weight
liver	3.39
kidney	0.77
fat	5.0
heart	0.33
lung	0.37
muscle	35.36
brain	0.6
blood	7.40
rest of body	46.78
Cardiac output	1.57 (ml/min·g) BW(g) ^{0.75}
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Blood flows (ml/min)	Percentage of Cardiac Output
liver	15.73
kidney	13.13
fat	6.56
heart	4.73
lung	100
muscle	26.11
brain	2.21
blood	100% = 1.57 (ml/min·g) BW(g) ^{0.75}
rest of body	31.53
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Partition Coefficients	
Blood:air	19.6
Fat:blood	152.5
Lung:blood	2.47
Liver:blood	5.25
Muscle:blood	2.98
Brain:blood	4.37
Heart:blood	2.68
Kidney:blood	4.45
Rest of body:blood	2.98
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Metabolism constants	
V _{max} (μg/min)	0.15
K _m (μg/ml)	0.019

Table 3
Pharmacokinetic Parameters in the Rat Following
a 2 hr Inhalation Exposure to 500 ppm PCE¹

Tissue	Area under curve ($\mu\text{g}\cdot\text{min}/\text{ml}$)	Biological half-life (min)	C _{max} ($\mu\text{g}/\text{g}$)
Liver	31247	423	152.4
Kidney	25868	425	107.5
Fat	1493190	578	1536.3
Heart	23179	328	106.6
Lung	18596	406	94.6
Muscle	24458	335	87.3
Brain	32975	455	173.9
Blood	8464	322	44.9

¹Each value represents the mean for 5 rats and 16 time points ranging from 15 minutes after the initiation of exposure to 72 hrs post-exposure.

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INDEX TERMS

Perchloroethylene

Physiologically-based Pharmacokinetic Model

Tissue concentration

Intraarterial administration

Inhalation Exposure

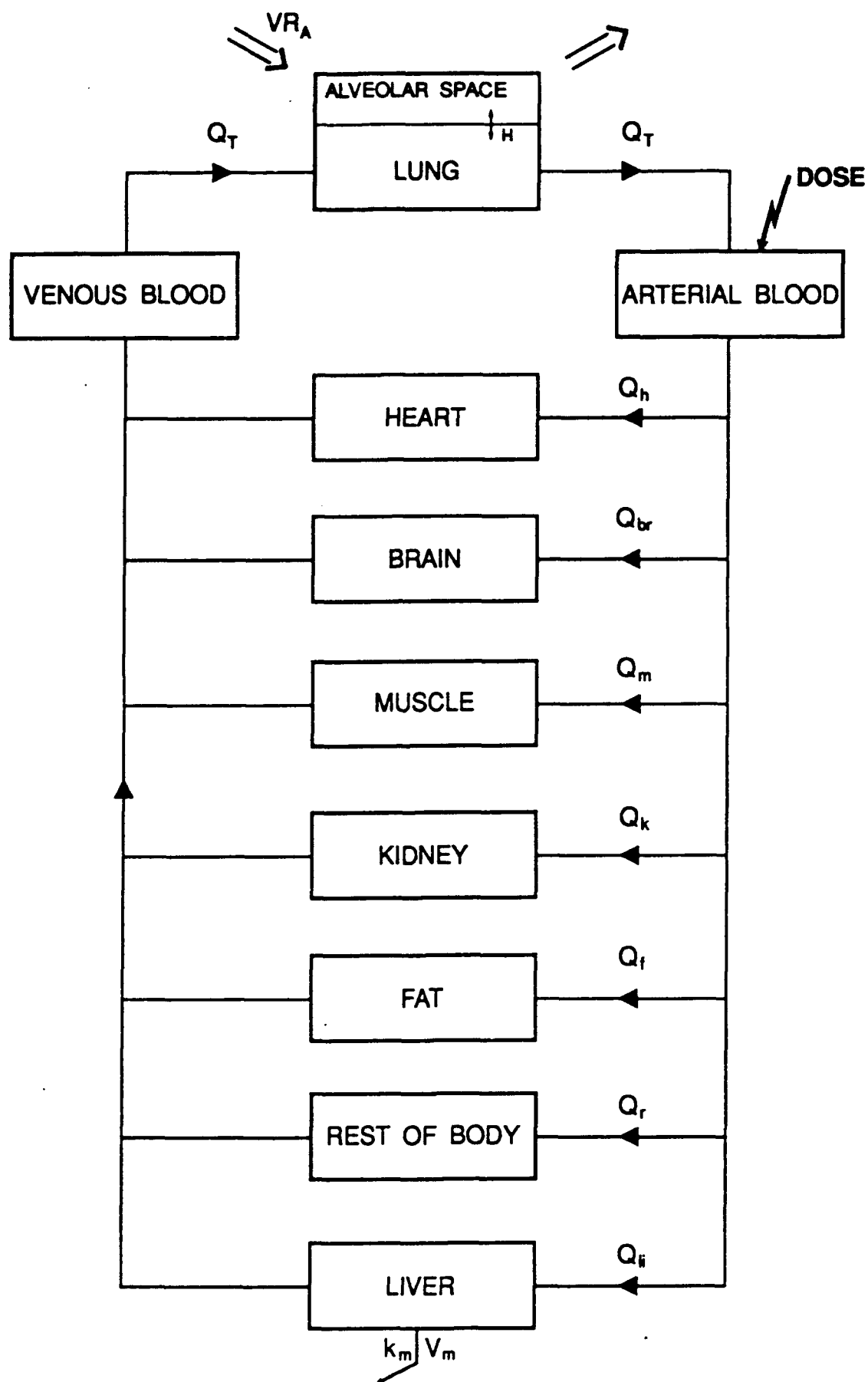
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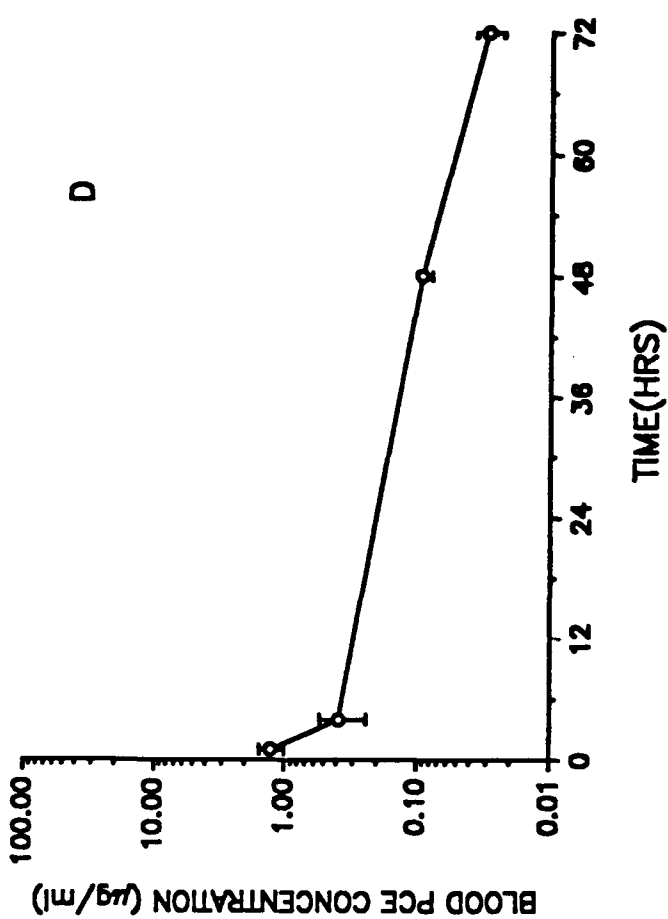
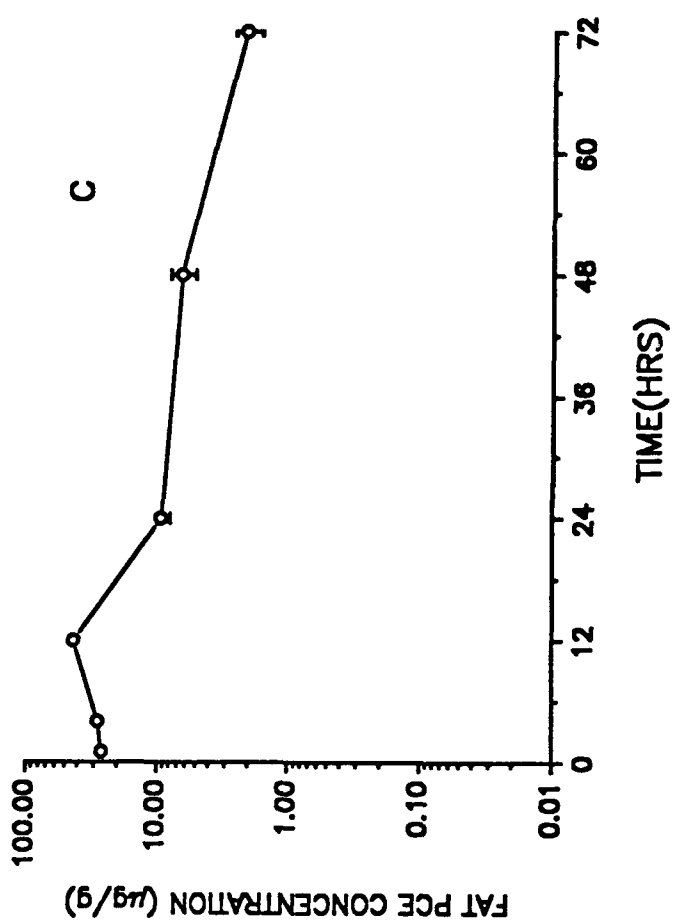
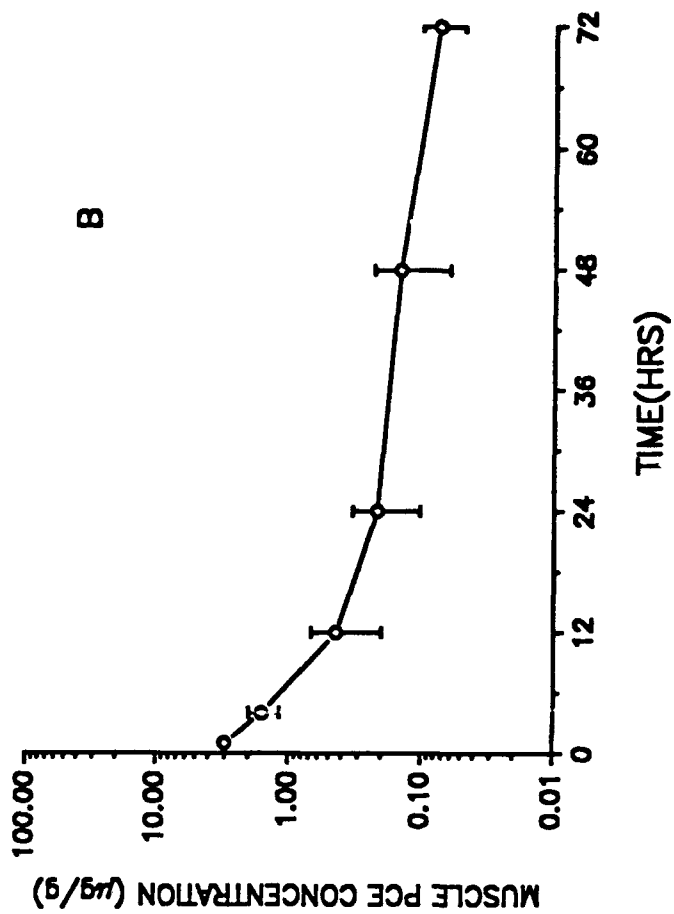
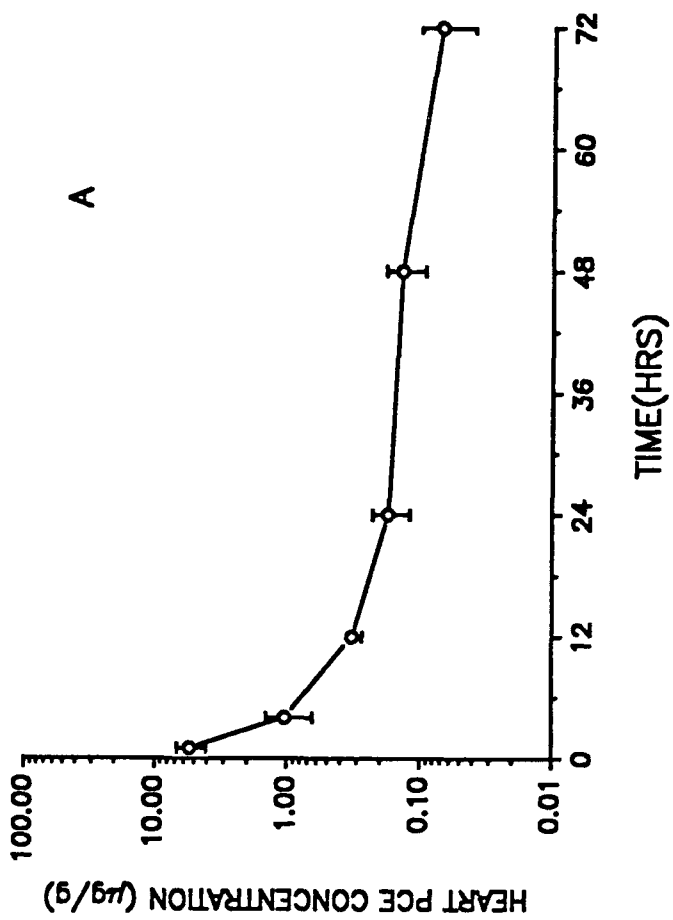
- ¹ Research sponsored by the Air Force Office of Scientific Research, Air Force Systems Command, USAF, under grant numbers AFOSR 870248 and 910356. The US Government is authorized to reproduce and distribute reprints for Governmental purposes notwithstanding any copyright notation thereon. This manuscript is submitted for publication with the understanding that the US Government is authorized to reproduce and distribute reprints for Governmental purposes.
- ² Presented in part at the 30th Annual Meeting of the Society of Toxicology, Washington, DC, February 1991.
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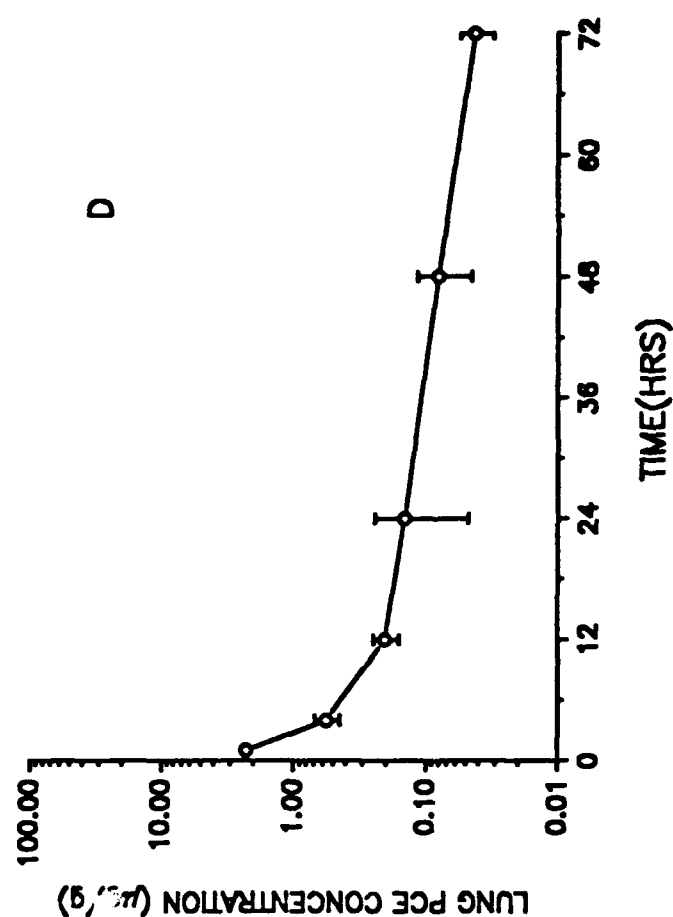
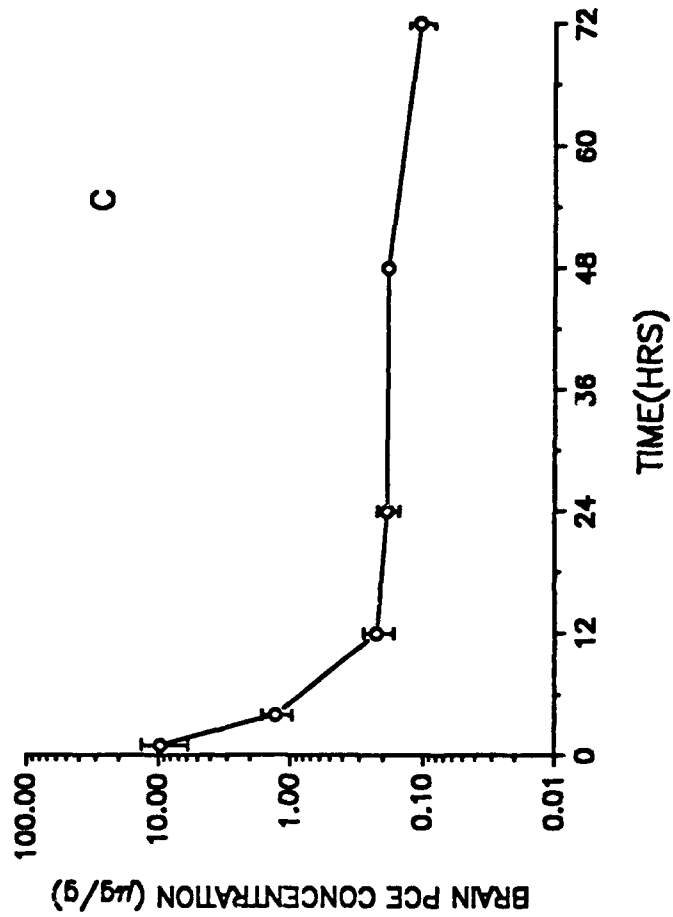
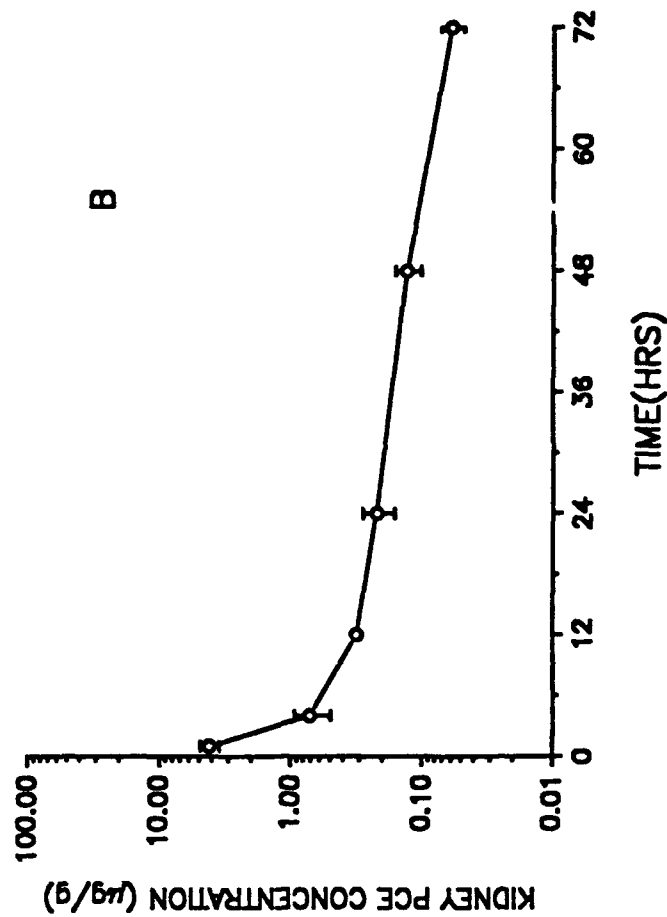
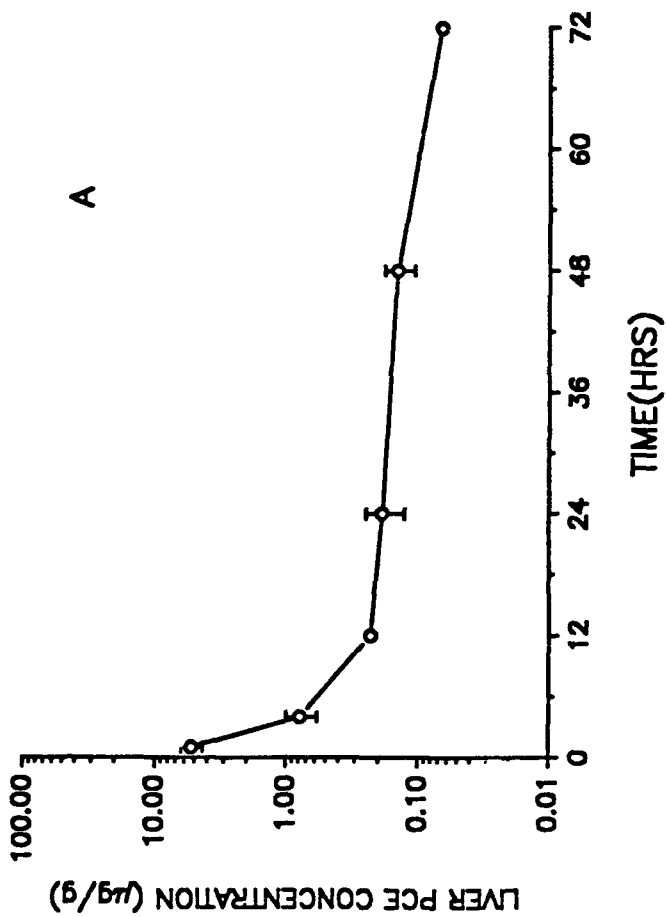
FIGURE LEGENDS

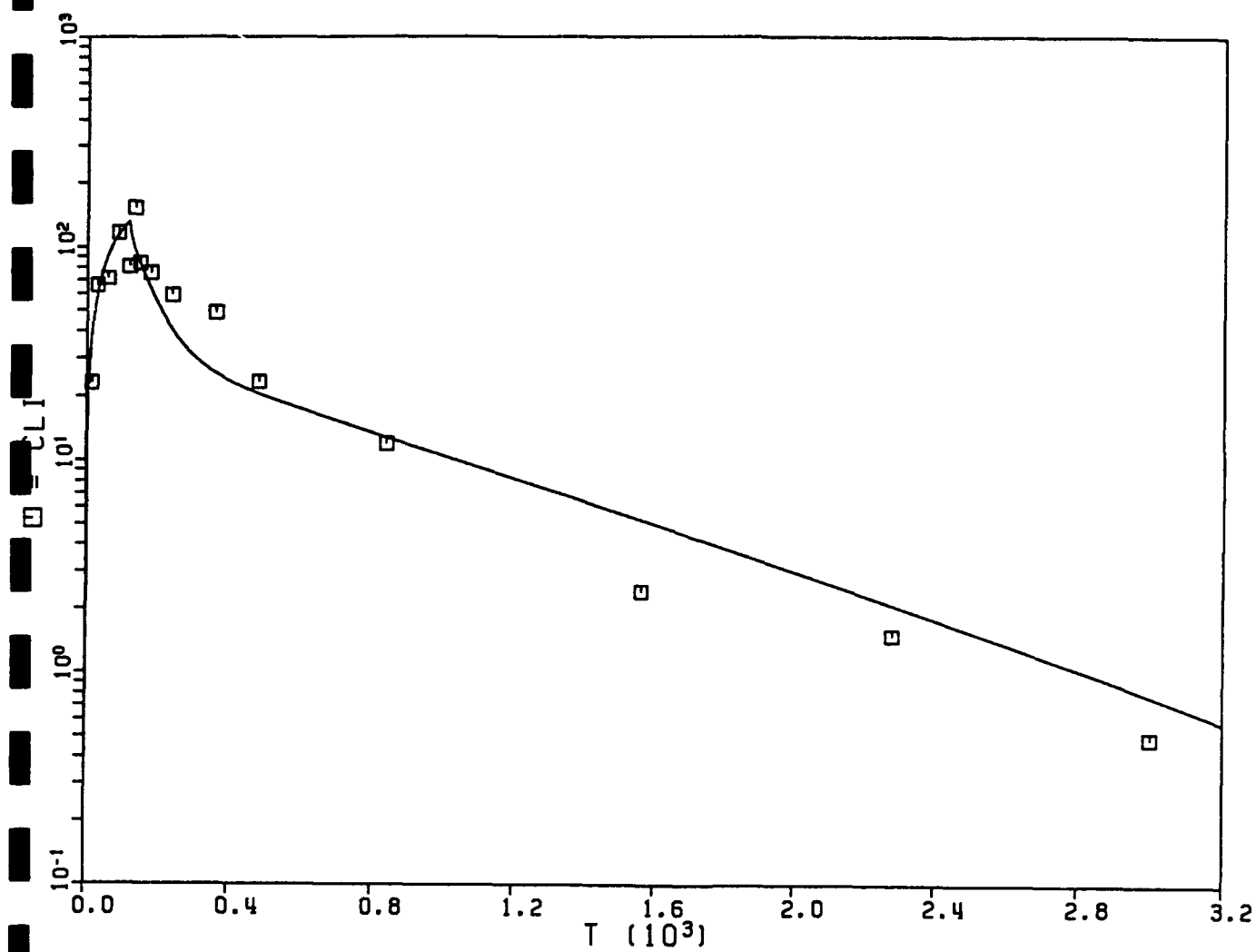
1. Diagram of the physiological pharmacokinetic model used to simulate the blood and tissue disposition of PCE during and following inhalation exposures in rats. The parameters used to describe the model are included in Table 2.
2. PCE concentrations measured in the liver, kidney, brain, and lung of rats following injection of a single bolus of 10 mg/kg of PCE through an indwelling carotid arterial cannula. Each point represents the mean \pm SE values for 4 rats.
3. PCE concentrations measured in the heart, muscle, fat and blood of rats following injection of a single bolus of 10 mg/kg of PCE through an indwelling carotid arterial cannula. Each point represents the mean \pm SE values for 4 rats.
4. Observed (\square) and model-predicted (-) PCE concentrations in the liver, kidney, brain, and lung of rats during and following 2 hr inhalation exposures to 500 ppm PCE in rats. Each point represents the mean value for 4 rats.
5. Observed (\square) and model-predicted (-) PCE concentrations in the heart, muscle, fat and blood of rats during and following 2 hr inhalation exposures to 500 ppm PCE in rats. Each point represents the mean value for 4 rats.

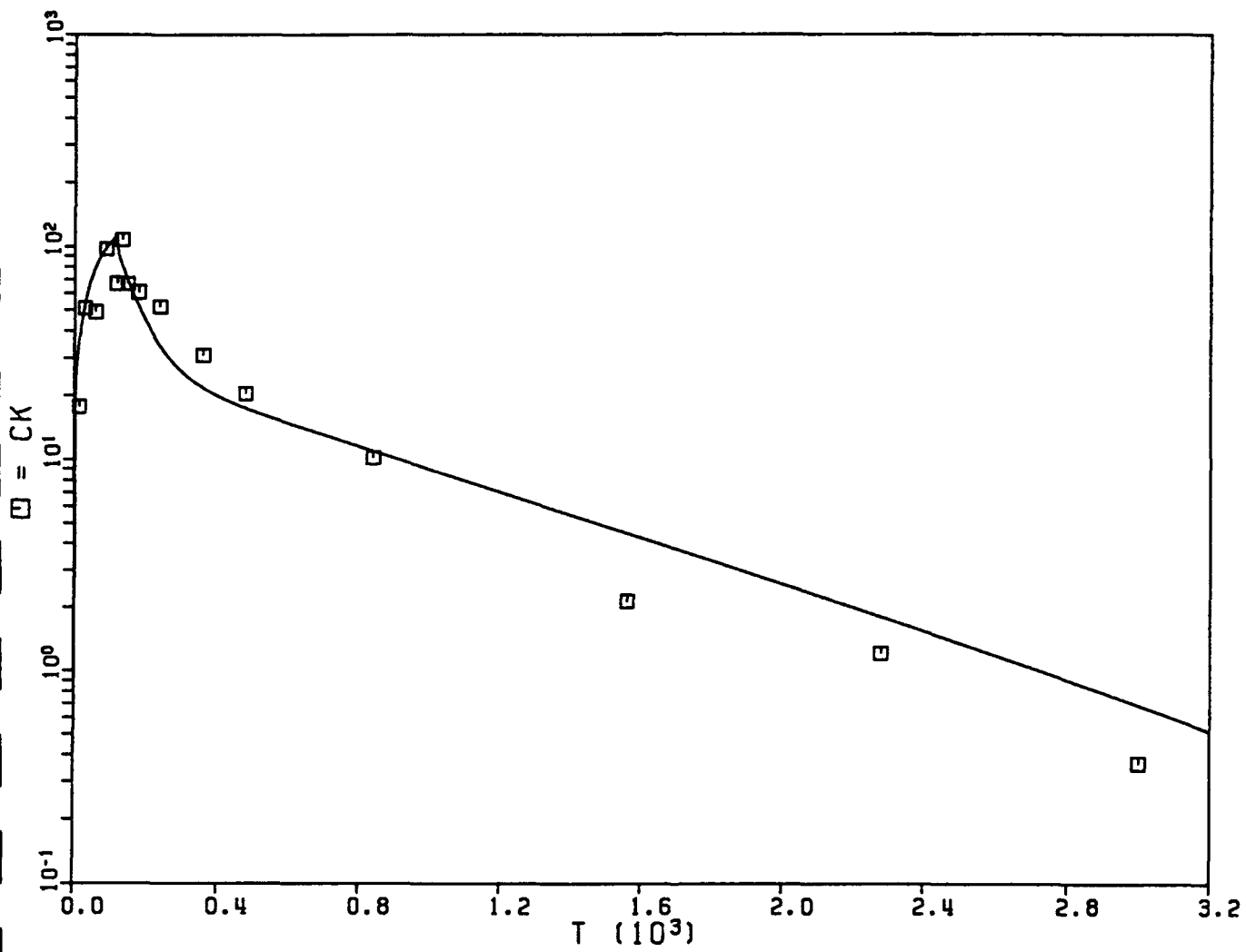
PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL OF PCE INTRAARTERIAL ADMINISTRATION

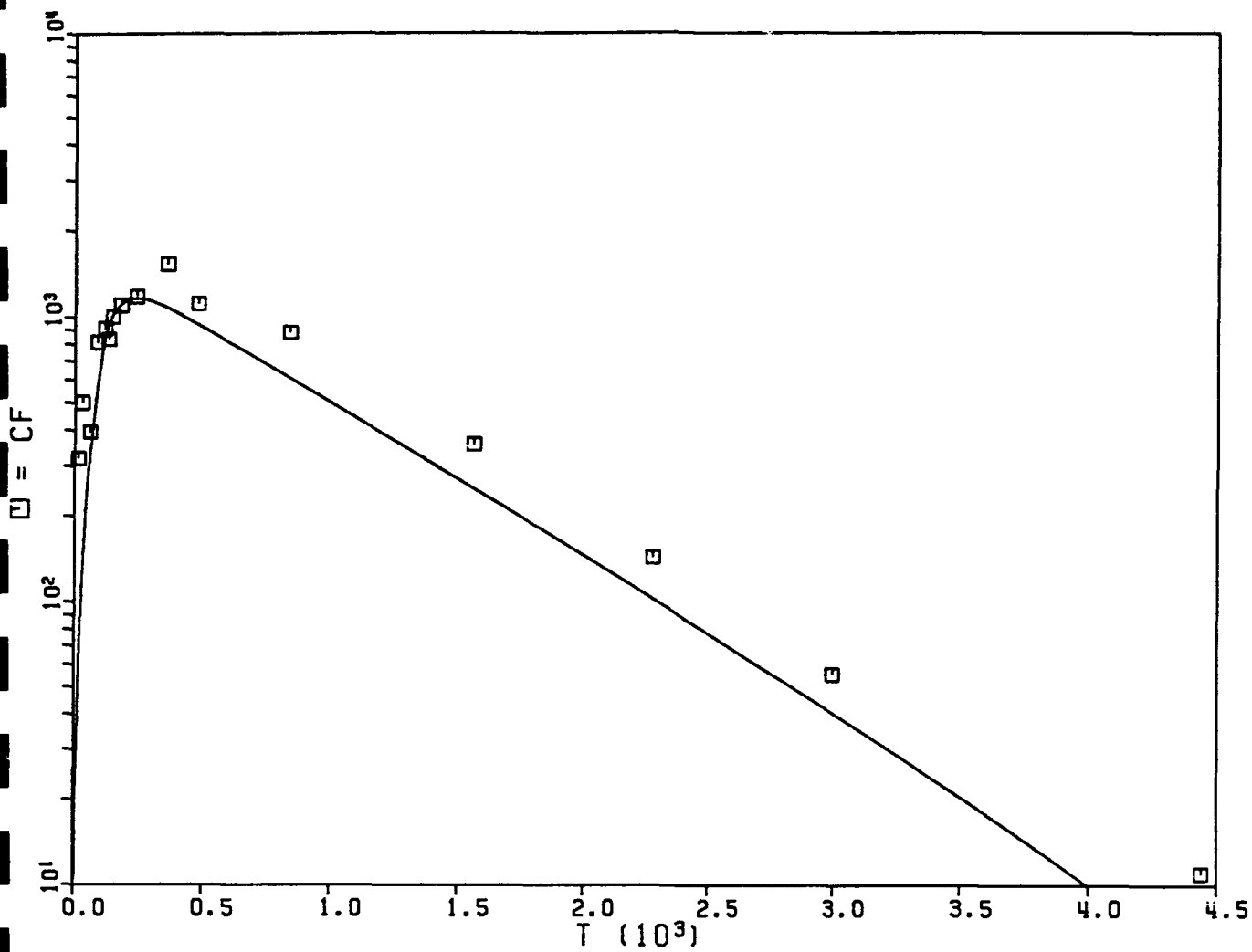


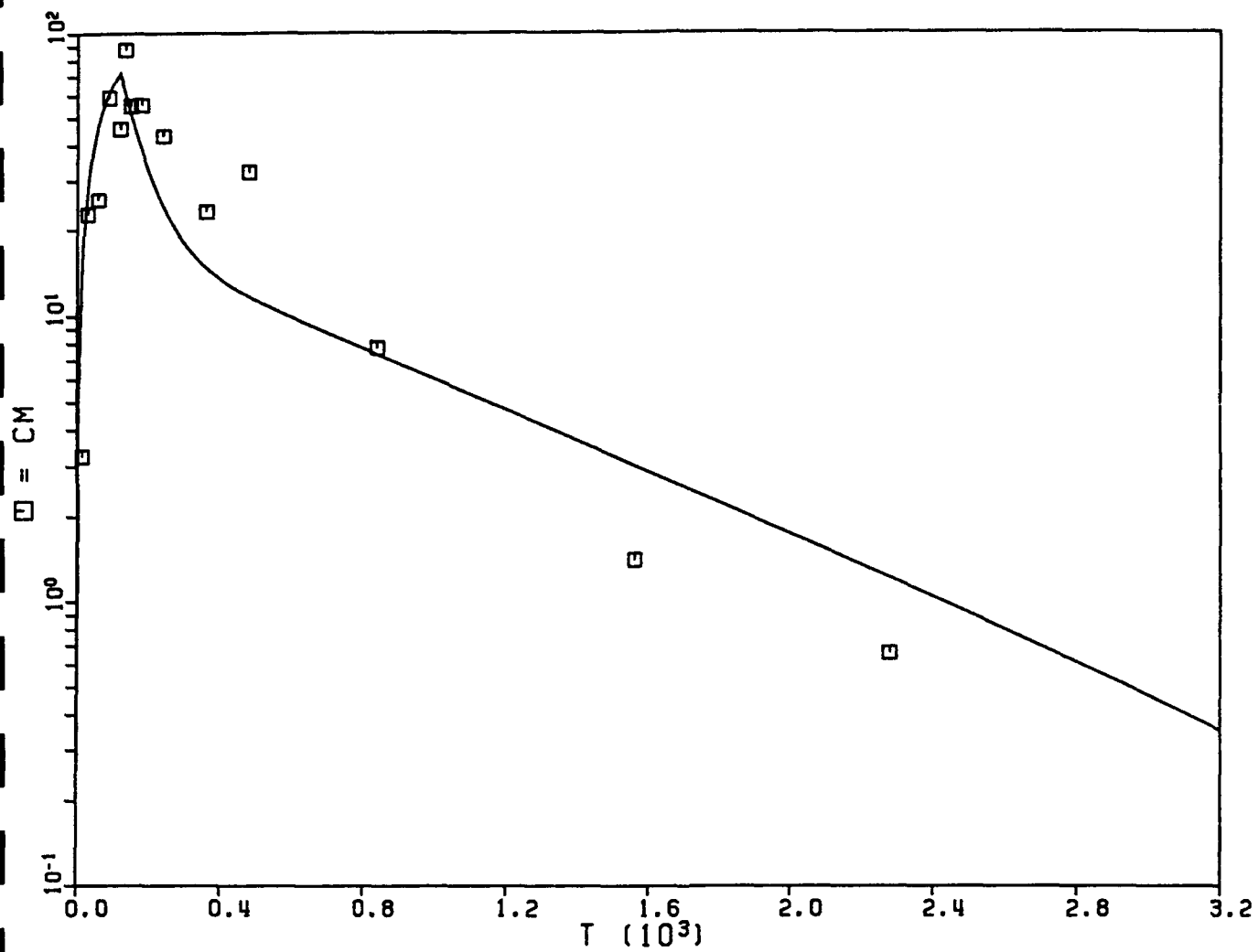


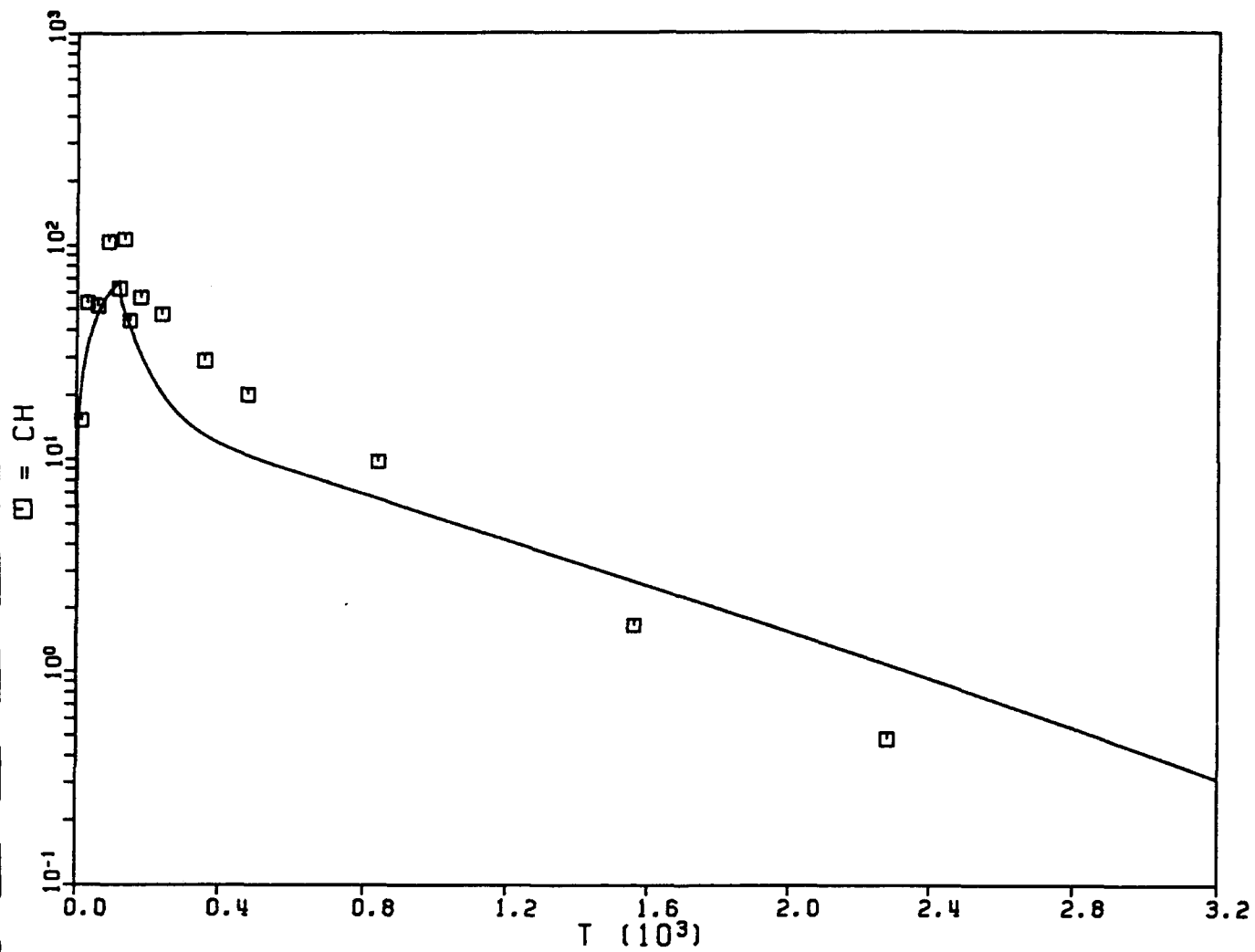


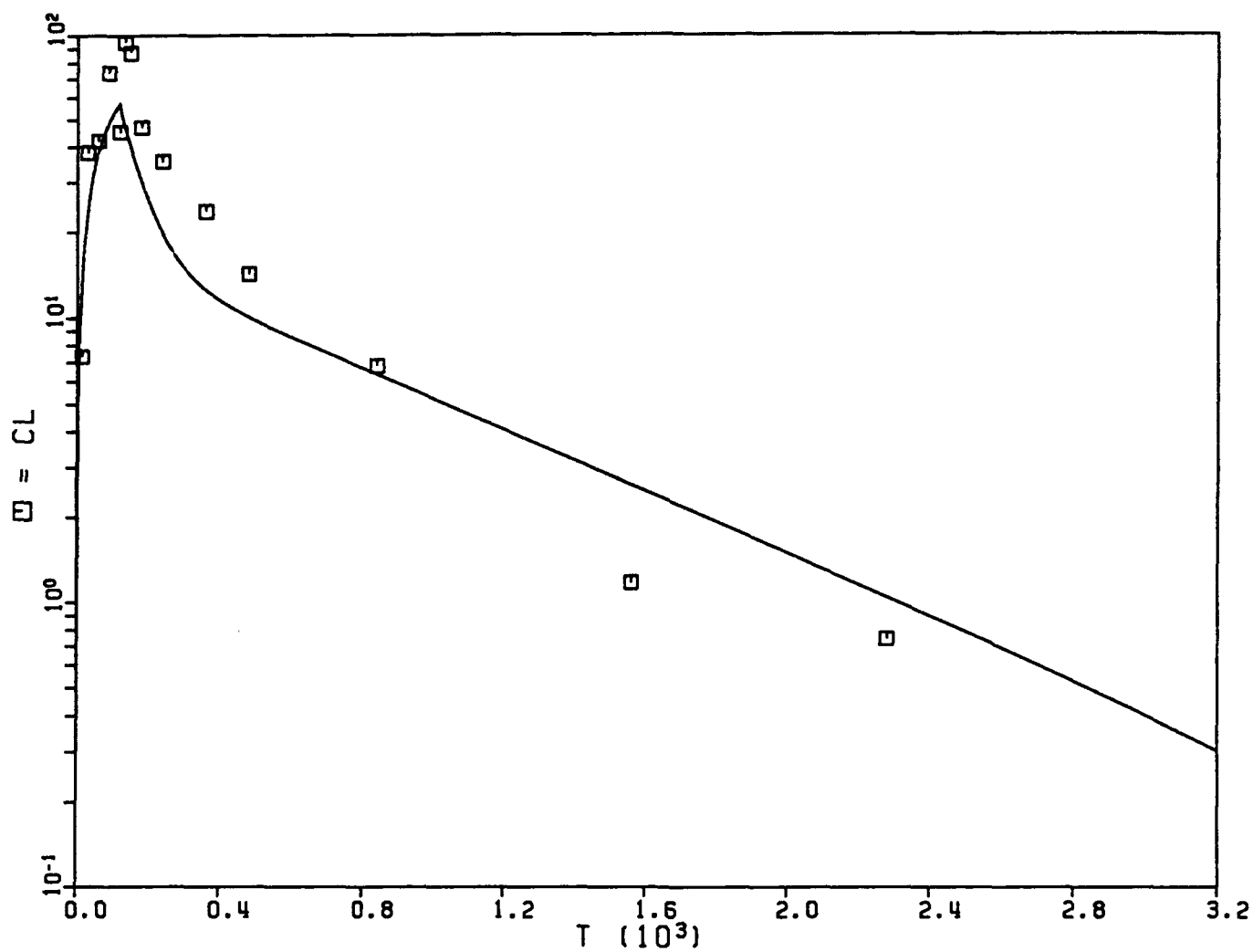


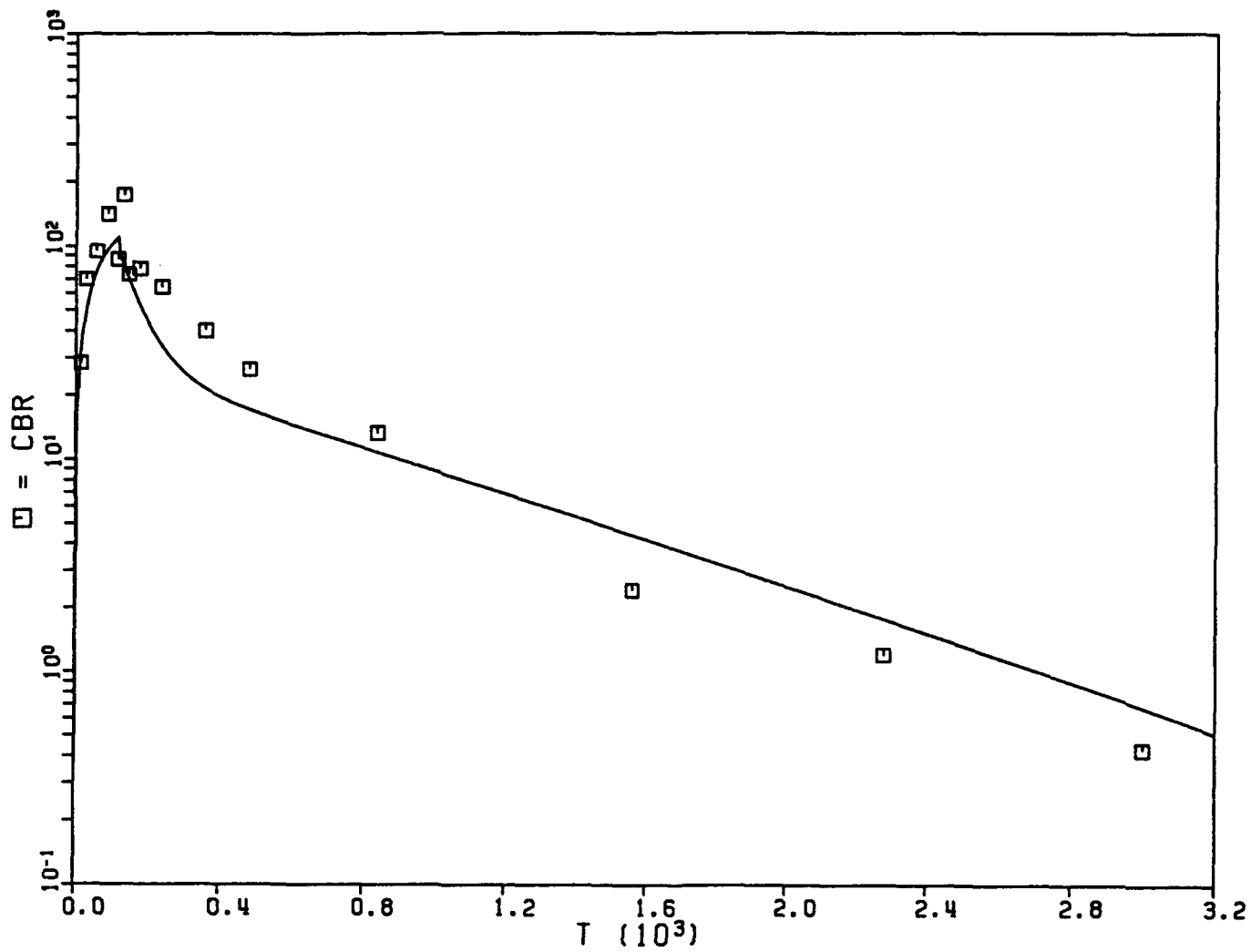


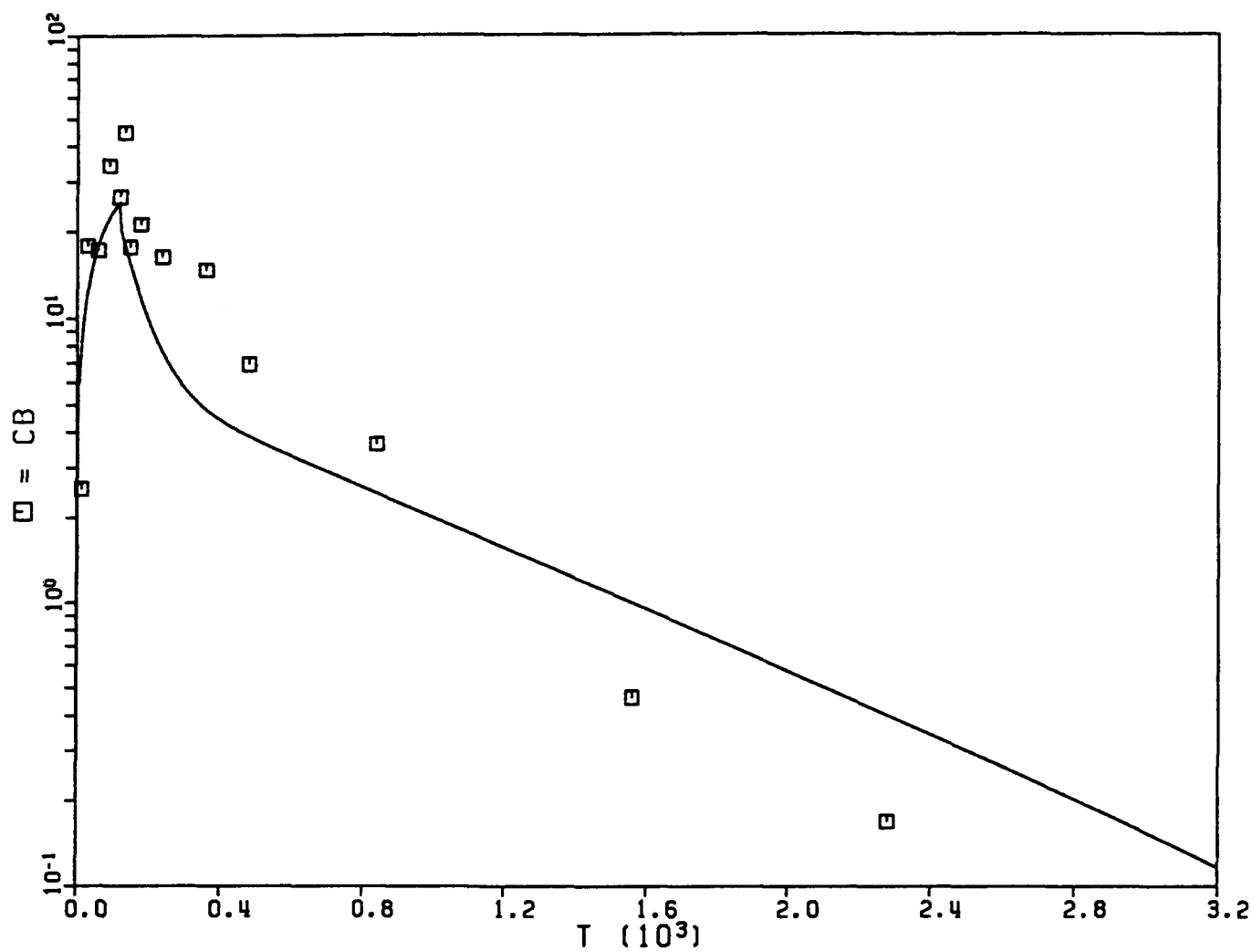












APPENDIX

SECTION C

Manuscript now being submitted to
Toxicology and Applied Pharmacology

Characterization of Perchloroethylene Uptake and Respiratory Elimination
for Evaluating Physiologically Based Model Predictions

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Abbreviated title: Physiological Model for Perchloroethylene

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ABSTRACT

Characterization of Perchloroethylene Uptake and Respiratory Elimination for Evaluating Physiologically Based Model Predictions. DALLAS, C.E., MURALIDHARA, S., CHEN, X.M., RAMANATHAN, R., VARKONYI, P., GALLO, J.M., and BRUCKNER, J.V. (1993) Toxicol. Appl. Pharmacol. 0, 00-00.

The pharmacokinetics of perchloroethylene (PCE) was studied in male Sprague-Dawley rats to characterize systemic uptake and respiratory elimination by direct measurements of the inhaled compound. Fifty or 500 ppm PCE was inhaled for 2 hr through a one-way breathing valve by unanesthetized male Sprague-Dawley rats of 325-375 g. Serial samples of separate inhaled and exhaled breath streams, as well as arterial blood, were collected during and following PCE inhalation and analyzed by headspace gas chromatography (GC). PCE exhaled breath and alveolar concentrations increased rapidly to near steady-state within about 60 min, and were directly proportional to the exposure concentration. Uptake of PCE in the blood was also rapid, but blood levels continued to increase progressively over the course of the 2-hr exposure at both dose levels. Cumulative uptake, or total absorbed dose, was proportional to the inhalation exposure level. Parameters for a blood flow limited physiologically-based model were determined from tissue concentration-time data in a companion publication, and a model characterized with PCE eliminated in the exhaled breath and to a limited extent by liver metabolism. PCE concentrations in the blood and exhaled breath were well predicted by the PBPK model. The usefulness of model simulations in predicting systemically absorbed doses of PCE was demonstrated, which can have utility in risk assessments involving the internal dose of volatile organics.

INTRODUCTION

Perchloroethylene (1,1,2,2-tetrachloroethylene, PCE) is a volatile organic compound (VOC) which is used in large quantities in industry for metal degreasing, dry cleaning fabrics and textiles, and as an intermediate for the production of other chemicals. Approximately 500,000 workers in the United States are estimated to be at risk of occupational exposure to PCE (NIOSH, 1978). Measurements of workplace air concentrations of PCE in the dry cleaning industry have determined mean time-weighted-averages (TWA, 8 hr), from 28.2 to 88.2 ppm (Materna, 1985), 4.0-149 ppm (Ludwig et al., 1983), and as high as 178 ppm (HSDB, 1987). Elevated concentrations of PER in indoor air have also been reported for residences with PER-contaminated water supplies (Highland et al., 1985; Andelman, 1985). Central nervous system (CNS) effects such as dizziness, headache, sleepiness, and incoordination have been reported in humans from acute PCE exposures of 100 to 200 ppm and above (Hake and Stewart, 1977; Stewart et al., 1970). Acute inhalation exposures to PCE in animals have also been reported to result in mild hepatotoxicity (Kylin et al., 1963), biochemical changes in the brain such as reduced RNA content (Savolainen et al., 1977), and CNS effects (Rowe et al., 1952; Goldbert et al., 1964).

Specific objectives of this study were to: 1) provide direct measurements of the respiratory uptake and elimination of PCE during and following inhalation exposures by simultaneously measuring PCE in the blood and exhaled breath; 2) determine the total dose of PCE absorbed systemically during 2-hour inhalation exposures using inhaled and exhaled breath determinations and the monitored volumes of respiration; 3) develop a physiologically-based pharmacokinetic (PBPK) model for PCE inhalation using parameters derived from tissue concentration-time data and evaluate the utility of the model using experimentally observed values.

MATERIALS AND METHODS

Animals. Adult, male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and dark from 1900 to 0700 hr. They were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow® were provided *ad libitum*. The rats were used after at least a 14-day acclimation period, at which time their body weight ranged from 325-375 g. Solvent exposures were initiated at approximately the same time each day (1000 to 1200 hr).

Test Material. Perchloroethylene (PER), of 99% purity, was obtained from Aldrich Company Inc. (Milwaukee, WI). The purity of the solvent was verified by gas chromatography.

Animal Preparation. An indwelling carotid artery cannula was surgically implanted into each animal. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml):acepromazine maleate (10 mg/ml):xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v/v/v). The cannulated animals were maintained in a harness and pulley system that allowed relative freedom of movement in metabolism cages during a 24-hr recovery period.

Inhalation Exposures. Each cannulated rat was placed into a restraining tube for nose-only inhalation exposure chambers (Battelle-Geneve, Switzerland). A face mask designed to fit the rat was held firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. A miniaturized one-way breathing valve (Hans Rudolph, Inc., St. Louis, MO) was attached to the face mask so that the valve entry port was directly adjacent to the nares of the test animal. This established separate and distinct airways for

the inhaled and exhaled breath streams with no significant mixing of the inhaled and exhaled air. The use of such a device for pharmacokinetic studies of inhaled halocarbons in small animals has been described in detail (Dallas et al., 1986). A known concentration of PCE was generated within a 70-liter gas sampling bag (Calibrated Instruments, Ardsley, NY) by injecting the appropriate quantity of the solvent into the bag filled with air. Uniform dispersion of the vapor was insured by a magnetic stirring bar within the bag. The bag was then connected in series by Teflon® tubing with a pneumotachograph, the one-way breathing valve and an empty 70-liter gas collection bag. The latter bag served as a reservoir to collect exhaled gas. Inhalation and exhalation sampling ports were located immediately adjacent to the breathing valve. Each cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposure chambers (Battelle-Geneve, Switzerland). A face mask with the breathing valve was held firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. PCE inhalation exposures of 2 hr duration were initiated only after stable breathing patterns were established. During this exposure period and for up to 4 hr afterward, serial inhaled and exhaled breath samples were taken at approximately the same time as blood samples from the carotid artery cannula. Both air and blood samples were then analyzed for PCE content by headspace gas chromatography.

Respiratory Measurements and Calculations. In order to calculate the total received dose of PCE during inhalation exposures, the respiration of each animal was continuously monitored. The respiratory monitoring technique was conducted according to the methods previously published in solvent exposure studies by this laboratory (Dallas et al., 1983, 1986, 1989). The airflow created by the animal's inspiration was recording both during and following PCE inhalation

exposure in terms of minute volume (volume of respiration per minute, V_E), respiratory rate (f), and tidal volume (V_T). An average value for these parameters for each individual animal was obtained by averaging the measurements taken at 15-min intervals during the 2-hr exposure. The mean \pm SD of these average values for the 500 ppm exposure group ($n=6$) were: $V_E = 189 \pm 21.5$; $f = 119.1 \pm 22.4$; $V_T = 1.62 \pm 0.34$. The mean \pm SD of these average values for the 50 ppm exposure group ($n=6$) were: $V_E = 216 \pm 43.1$; $f = 134.5 \pm 14.9$; $V_T = 1.67 \pm 0.36$.

Calculations of PCE uptake and elimination were conducted utilizing the equations presented in a previous VOC inhalation study in rats (Dallas et al., 1989). Since the V_E and the exhaled breath PCE concentration at each sampling point were measured, subtraction of the quantity of PCE exhaled by the animal from the amount inhaled yielded an estimation of the quantity of PCE taken up during sequential sampling periods (cumulative uptake). The percent uptake during each exposure period was determined by dividing the cumulative uptake by the total inhaled dose for the time period.

A physiologically-based pharmacokinetic (PBPK) model was used to describe the disposition of PCE in the rat (Fig. 1). It was assumed that a blood flow-limited model was adequate to characterize the tissue distribution of PCE. Previous PBPK models for VOCs have utilized blood-flow-limited organ representations (Ramsey and Anderson, 1984; Angelo and Pritchard, 1984; Dallas et al., 1989, 1991). Compartmental volumes and organ blood flows were obtained from the values determined in this laboratory for the rat (Delp et al., 1991). Alveolar ventilation was determined experimentally as 50% of the minute volume, accounting for deadspace in the animal and in the breathing valve. In vivo tissue:blood partition coefficients were calculated from tissue concentration-

time data for PCE following ia administration in a companion study (Dallas et al., 1993), using the area method of Gallo et al. (1987). The metabolic parameters K_m and V_{max} , and blood:air coefficients were estimated from the observed data for liver and blood by nonlinear regression analysis.

The alveolar mass transfer coefficient was based on the alveolar permeability-area product for methylene chloride (Angelo and Pritchard, 1987). The lung:air partition coefficient was derived using the AUC method by Gallo et al. (1985). Differential mass balance equations, incorporating the parameters listed in Table 1, that described the transport of PCE in the rat were numerically integrated with the Advanced Continuous Simulation Language (ACSL) computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted PCE blood and tissue concentrations over time. The model-predicted cumulative uptake values were the sum of the simulated amounts of PCE in each tissue compartment in the model.

Analysis of PCE in air and blood. The concentration of TCE in the inhaled and exhaled air samples collected during and following the inhalation exposure were measured with a Tracor MT560 gas chromatograph (Tracor Instruments, Austin, TX). Standards were prepared in each of four 9-liter bottles equipped with Teflon® stoppers with needles from which air samples could be taken by syringe. Analyses for the 500 ppm exposures were conducted using a flame ionization detector (FID), while the analyses for the 50 ppm inhalation exposures were conducted using an electron capture detector (ECD). In either case, air samples were procured with a gas-tight, 1-ml syringe and injected directly onto an 8-ft X 1/8-in stainless steel column packed with 0.1% AT 1000 on GraphPak. Operating temperatures were: 150°C, injection port; 200°C, FID detector; 350°C, ECD detector; 110°C, isothermal column operation. When using the ECD, gas flow rates

of 40 ml/min were employed for nitrogen (carrier gas), with an additional make-up gas flow rate to the detector of 30 ml/min.

Blood levels of PCE were measured by gas chromatographic headspace analysis. Blood samples were withdrawn from the arterial cannula via a stopcock into a 1-ml syringe. Depending on the anticipated blood TCE concentration, from 25 to 200 μ l for the blood was taken from the stopcock with an Eppendorf pipette and transferred to chilled headspace vials (Perkin-Elmer, Norwalk, CT). These vials were capped immediately with PTFE-lined butyl rubber septa and washers and tightly crimped. Each sample vial was then placed into the HS-6 auto-sampler unit of a SIGMA 300 gas chromatograph (Perkin-Elmer, Norwalk, CT), where it was heated to a present temperature by a high precision thermostat device. A predetermined volume of the vapor was then injected automatically into the column for analysis. The column used was an 8-ft X 1/8-in stainless-steel column packed with 10% FFAP chromasorb W-AW (80-100 mesh). Operating temperatures were: 200°C, injection port; 400°C, ECD detector; and 110°C column oven. The carrier gas was 5% argon-methane, at a flow rate of 40 ml/min, with a make-up gas flow rate of 20 ml/min to the detector.

RESULTS

Actual PCE concentrations inhaled by the animals were determined by analysis of air samples taken from the airway immediately adjacent to the breathing valve. Inhaled PCE concentrations for the six rats in each group were 52.8 ± 2.2 ppm ($\bar{x} \pm SD$) for the 500 ppm exposure and 53.1 ± 5.1 ppm ($\bar{x} \pm SD$) for the 50 ppm exposures, and were comparable to the desired inhalation concentrations of 50 and 500 ppm.

Significant respiratory elimination of unchanged PCE was evident during the inhalation exposure period, with near steady-state PCE levels achieved in the exhaled breath within 20-30 min. These near-steady state concentrations were approximately 2.1-2.4 $\mu\text{g/ml}$ in the exhaled breath of the 500 ppm exposed rats (Fig. 2b). In the 50 ppm inhalation exposure group, these exhaled breath levels at near-steady state were in the range of 0.20-0.22 $\mu\text{g/ml}$ (Fig. 3b).

PCE was rapidly absorbed from the lungs with relatively high arterial blood concentrations of PCE achieved at the first sampling time (i.e., 2 min). Unlike the exhaled breath data, the concentration of PCE in the blood progressively increased over the course of the 2-hr exposure in both exposure groups. The rate of increase in PCE concentration in the blood was greater in the 500 ppm (Fig. 2a) than in the 50 ppm group (Fig. 3a). Arterial blood PCE concentrations were not proportional to the inhaled concentration. After the initial rapid uptake phase over the first 30 minutes of exposure, blood levels in the 500 ppm rats were 12 to 17 times higher than 50 ppm rats. Upon cessation of PCE inhalation, the chemical was rapidly eliminated. As can be seen in Figs. 2 and 3, PCE concentrations in blood and in exhaled breath declined rapidly.

Measurement of the total cumulative uptake of PCE was made by accounting for the quantity of unchanged PCE that was exhaled during the inhalation exposure period. The total cumulative uptake of PCE from the 2-hr exposure to 500 ppm (Fig. 4a) was $28.1 \pm 4.3 \text{ mg (x} \pm \text{SD)}$, or 79.9 mg/kg bw. The 2-hr exposure to 50 ppm PCE (Fig. 4b) resulted in a cumulative uptake of $3.9 \pm 0.9 \text{ mg (x} \pm \text{SD)}$, or 11.2 mg/kg bw. Percent systemic uptake of PCE is shown in Figure 5. Percentage uptake was relatively constant after the first 20 minutes of inhalation at about 50% for 50 ppm exposure and approximately 40% for 500 ppm exposure.

The PBPK model predictions of blood and exhaled breath concentrations of PCE are shown in Figures 2 and 3. Both observed and predicted exhaled breath concentrations of PCE rapidly achieved a steady state following the initiation of exposure. Predicted concentrations of PCE in the exhaled air both during and following 500 ppm (Fig. 2a) and 50 ppm (Fig. 2b) inhalation exposure agreed well with the observed concentrations. The steep increase in blood concentration of PCE during 500 ppm inhalation exposure (Fig. 2a) was accurately predicted by the model simulations. PCE blood concentrations post-exposure were also well simulated. The rapid uptake of PCE in the blood during the first hour of 50 ppm inhalation (Fig. 2b) was slightly over predicted, but the observed values during the second half of the exposure period and following exposure were approximately one-half of the predicted values.

The PBPK model was also utilized to generate predictions of cumulative and percent uptake of PCE during inhalation exposure, which were compared to values calculated from direct measurements. Cumulative uptake of PCE was well predicted over the course of the 500 ppm inhalation exposure (Fig. 4a). During 50 ppm exposure the magnitude of the degree of underprediction by the model increased steadily over the course of the 2 hr exposure, resulting in 20% less than the observed value at 120 minutes (Fig. 4b). In the second hour of 500 ppm exposure model predictions of percent uptake were within 2% of the observed values (Fig. 5a). For the 50 ppm exposure, the predicted percent uptake was consistently about 5% below the observed percent uptake (Fig. 5b).

Model predictions were also conducted of exhaled breath concentrations using different lung:blood partition coefficients, in order to determine the impact of this parameter on exhalation of PCE in species with different lung:blood partition coefficients. Employing the assumption of equivalent

tissue:blood coefficients, as well as the lung:blood partition coefficient between species, a new blood:air partition coefficient can be obtained as follows:

$$r_a = r_l r_{b:a}$$

where r_a = lung:air partition coefficient

r_l = lung:blood partition coefficient

$r_{b:a}$ = blood:air partition coefficient

In the current rat model, $r_a = 46.87$, $r_l = 2.48$, and $r_{b:a} = 18.9$. Changing $r_{b:a}$ to 10.3, the human value used by Bois et al. (1990) results in $r_a = 25.54$. Predictions of PCE exhalation over time were made using these two r_a values (i.e., 46.87 and 25.54), corresponding to the difference in coefficients between rats and humans. The exhaled PCE concentration values were compared at the time points of 1 hr and 3 hr (Table 3), and the predicted exhaled concentration time course is shown for 500 ppm (Fig. 6a) and 50 ppm (Fig. 6b). It can be observed that even an approximate doubling in the magnitude of the lung:blood partition coefficient results in only a small difference in the exhaled concentration at either inhalation concentration. The larger r_l value resulted in a slightly smaller exhaled concentration.

DISCUSSION

As is characteristic for many volatile halocarbons, the major route of elimination for PCE in laboratory animals and man is by exhalation of the parent compound. As with trichloroethylene (TCE) and trichloroethane (TRI), the pharmacokinetic data base for PCE is unique in that there have been several studies published with direct measurements of the elimination of the halocarbon in humans. This allows interspecies comparisons of the quantity of the

halocarbon eliminated in the exhaled breath between laboratory animals and man. Fernandez et al. (1976) measured PCE in the exhaled breath of human volunteers following 1 to 8 hours exposure in dynamic exposure chambers. Following exposure to 100 ppm for 2 hours, the PCE concentration in the exhaled breath at 1 and 2 hrs post-exposure were 0.06 and 0.047 $\mu\text{g}/\text{ml}$. Assuming a linear scale up from the 50 ppm data in the current investigation to 100 ppm inhalation concentration, the expired air PCE concentration in the rats at these two time points would be 0.07 and 0.04 $\mu\text{g}/\text{ml}$, respectively. Assuming a linear scaleup of the exposure concentration to that used in the current study, the postexposure PCE exhaled breath levels in several other human studies (Stewart et al., 1961; Stewart et al., 1970) were also similar in magnitude to the measurements of PCE in the expired air of rats in this current study. The similar pattern of concentrations of halocarbon eliminated in the exhaled breath of rats and humans was also noted for TRI (Dallas et al., 1989) and TCE (Dallas et al., 1991). Since the blood:air partition coefficient for PCE, TRI, and TCE is markedly higher in rats than in humans (Gargas et al., 1989), it might be anticipated that this physicochemical difference would result in a greater concentration of halocarbon expired in humans relative to rats for an equivalent exposure. The PCE tissue:air coefficients for rats are also significantly higher than those published for humans (Bois et al., 1990).

Evaluation of the relative importance of the species differences in blood:air and tissue:air partition coefficients therefore is needed in order to determine why human and rat exhaled breath concentrations can be so close when these coefficients are so dissimilar. Considering the PBPK model for PCE by Bois et al. (1990), the difference between the rat and human blood:air partition coefficient is one of the primary differences in the models (beside the size and

the scaled parameters). Calculating the tissue:blood coefficient from these blood:air and tissue:air coefficients, however, reveals that the tissue:blood coefficients for rats and humans have been assumed as being practically equivalent for rats and humans. Therefore, while the blood:air partition coefficients between the two species are quite different, this is apparently based on the assumption that the tissue:blood partition coefficients are the same.

In the current paper the chemical input into the model occurs at the lung-air interface and is characterized by a mass transfer coefficient and lung:air partition coefficient. Since the value of the mass transfer coefficient would not be rate-limiting for chemical uptake, examination of changes in the lung:air partition coefficient on exhaled breath concentration were made. In order to examine the potential impact of interspecies differences in the lung:air partition coefficient (r_a) on model predictions of the respiratory elimination of PCE in rats and humans. Simulations were conducted with two different r_a values corresponding to differences between rats and humans in the parameter. This was the only parameter changed in the comparison. The differences between the exhaled concentrations predicted using the two different r_a values were very small, at either 50 or 500 ppm exposure. It could be anticipated, then, that the magnitude of PCE exhalation by humans and rats could also be similar even though the blood:air partition coefficients are so different.

There has been an emphasis in the application of PB-PK models to human risk assessment. The amount of PCE metabolized by animals that showed tumor responses in cancer bioassays was predicted using a PBPK model (Chen and Blancato, 1987). Scaling up the predictions of metabolite formation to humans and employing the dose response relationship in the animal studies was then used to calculate human

carcinogenic risk. In a unique application of an inhaled dose calculation, the respiratory exposure of humans to PCE in indoor air (resulting from PCE volatilized indoors from contaminated ground water) was determined and employed as the dose input for a PBPK model for PCE in humans (Bogen and McKone, 1988). Using a PBPK model for PCE as an example, Farrar et al. (1989) characterized the degree of uncertainty in the model output and human risk estimates due to uncertainty in model parameters. The rate of PCE metabolite formation in mice, rats, and humans was predicted using a PBPK model and compared to observed data available in the literature (Bois et al., 1990). Hattis et al. (1990) have compared the predictions of metabolite formation from PCE from the different published models. These studies did not make comparisons of the PBPK model predictions of parent compound uptake or disposition with experimentally derived data, as the emphasis was on metabolite formation and risk assessment.

Total cumulative uptake of the PCE over time was well simulated over the course of 500 ppm inhalation, and underpredicted during 50 ppm exposure. Predictions of unchanged PCE in the expired air of rats following exposure to inhaled PCE were well simulated by a PBPK model by Ward et al. (1988) in comparison to previously published data (Pegg et al., 1979). The authors increased the fat-air partition coefficient from the measured value of 1638 to 2300 in order to account for the concentrations of PCE in the post-exposure expired air. In the present investigation, the partition coefficient for fat:blood derived from the tissue area-under-the-concentration-time curve (AUC) values was 109, which corresponds to a fat:air coefficient of 2881. While published values of PCE exhaled air and blood concentrations were available during inhalation exposure in humans (Fernandez et al., 1976) for use in comparison to model predictions by Ward et al. (1988), no published values were

available for these parameters during PCE inhalation in rats. In the current investigation, exhaled air concentrations during exposure to PCE in rats were well predicted by the PBPK model employed. PCE determination in the blood of rats during inhalation exposure also were not previously available for verification of PBPK model predictions. Values for the uptake and elimination of PCE in rat arterial blood at the higher dose in the current study were similar in magnitude and pattern to the prediction by the PBPK model. The utility of the model for extrapolation to lower doses was not demonstrated for arterial blood, however, due to the overprediction in the 50 ppm exposure group.

The ability of the current PBPK model to predict disposition of PCE increases the confidence in the utility of the model in health risk assessments. The use of *ia* tissue concentration-time data for establishing PBPK model parameters was found to be helpful in developing a model to provide accurate predictions both during and following inhalation exposure. Studies of the disposition of PCE tissue concentration-time data during and following inhalation exposures would further expand the degree of certainty in model predictions for risk assessment purposes.

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Table 1
Parameters Used in the Physiologically Based
Pharmacokinetic Model for PCE in the Rat

Parameter	
Alveolar ventilation ml/min	108 (50 ppm); 94.5 (500 ppm)
Inhaled Gas Concentration (mg/ml)	0.35/(50 ppm); 3.55 (500 ppm)
Body Weight (kg)	340
Tissue volumes (ml)	Percentage of Body Weight
liver	3.39
kidney	0.77
fat	5.0
heart	0.33
lung	0.37
muscle	35.36
brain	0.6
blood	7.40
rest of body	46.78
Cardiac output	1.57 (ml/min•g) BW(g) ^{0.75}
Blood flows (ml/min)	Percentage of Cardiac Output
liver	15.73
kidney	13.13
fat	6.56
heart	4.73
lung	100
muscle	26.11
brain	2.21
blood	100% = 1.57 (ml/min•g) BW(g) ^{0.75}
rest of body	31.53
Partition Coefficients	
Blood:air	19.6
Fat:blood	152.5
Lung:blood	2.47
Liver:blood	5.25
Muscle:blood	2.98
Brain:blood	4.37
Heart:blood	2.68
Kidney:blood	4.45
Rest of body:blood	2.98
Metabolism constants	
Vmax (μg/min)	0.15
Km (μg/ml)	0.019

TABLE 3

Predicted PCE Exhaled Concentrations [$\mu\text{g/ml}$]
at Two Different r_a Values

r_a	$c_{inh} = 50 \text{ ppm}$		$c_{inh} = 500 \text{ ppm}$	
	$t = 1 \text{ hr}$	$t = 2 \text{ hr}$	$t = 1 \text{ hr}$	$t = 2 \text{ hr}$
46.87 (rat)	0.25	0.26	2.36	2.48
25.54 (human)	0.26	0.28	2.40	2.70

ACKNOWLEDGEMENT

The authors are grateful to Ms. Joy Wilson and Mrs. Judy Bates for their expertise in preparation of this manuscript, and to Miss Elizabeth Lehman for her collation and recording of data.

INDEX TERMS

Perchloroethylene

Physiologically-based Pharmacokinetic Model

Respiratory Elimination

Pharmacokinetics

Inhalation Exposure

FOOTNOTES

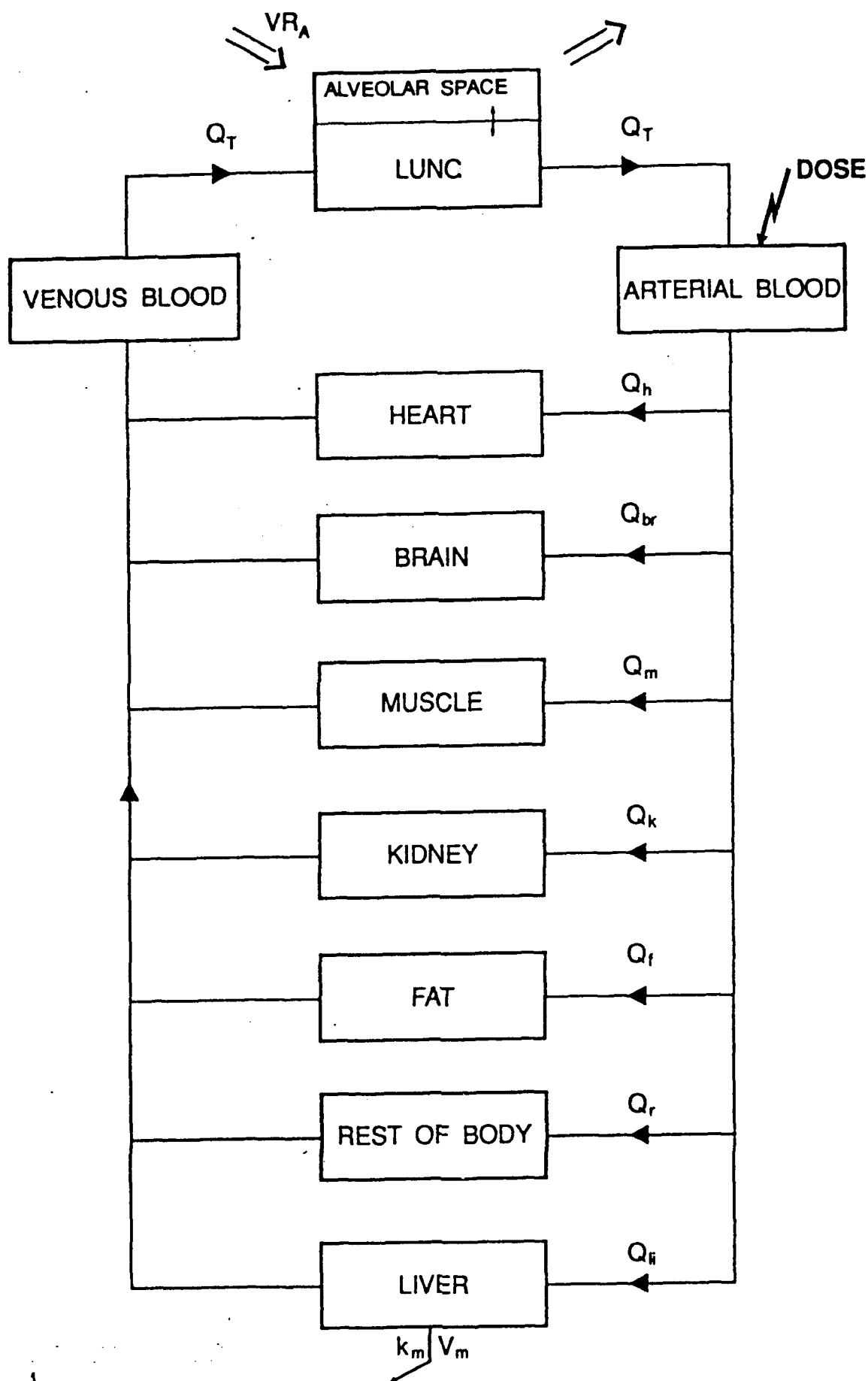
- ¹ Research sponsored by the Air Force Office of Scientific Research, Air Force Systems Command, USAF, under grant numbers AFOSR 870248 and 910356. The US Government is authorized to reproduce and distribute reprints for Governmental purposes notwithstanding any copyright notation thereon. This manuscript is submitted for publication with the understanding that the US Government is authorized to reproduce and distribute reprints for Governmental purposes.
- ² Presented at the 28th Annual Meeting of the Society of Toxicology, Washington, DC, February 1989.
- ³ To whom correspondence should be addressed.

FIGURE LEGENDS

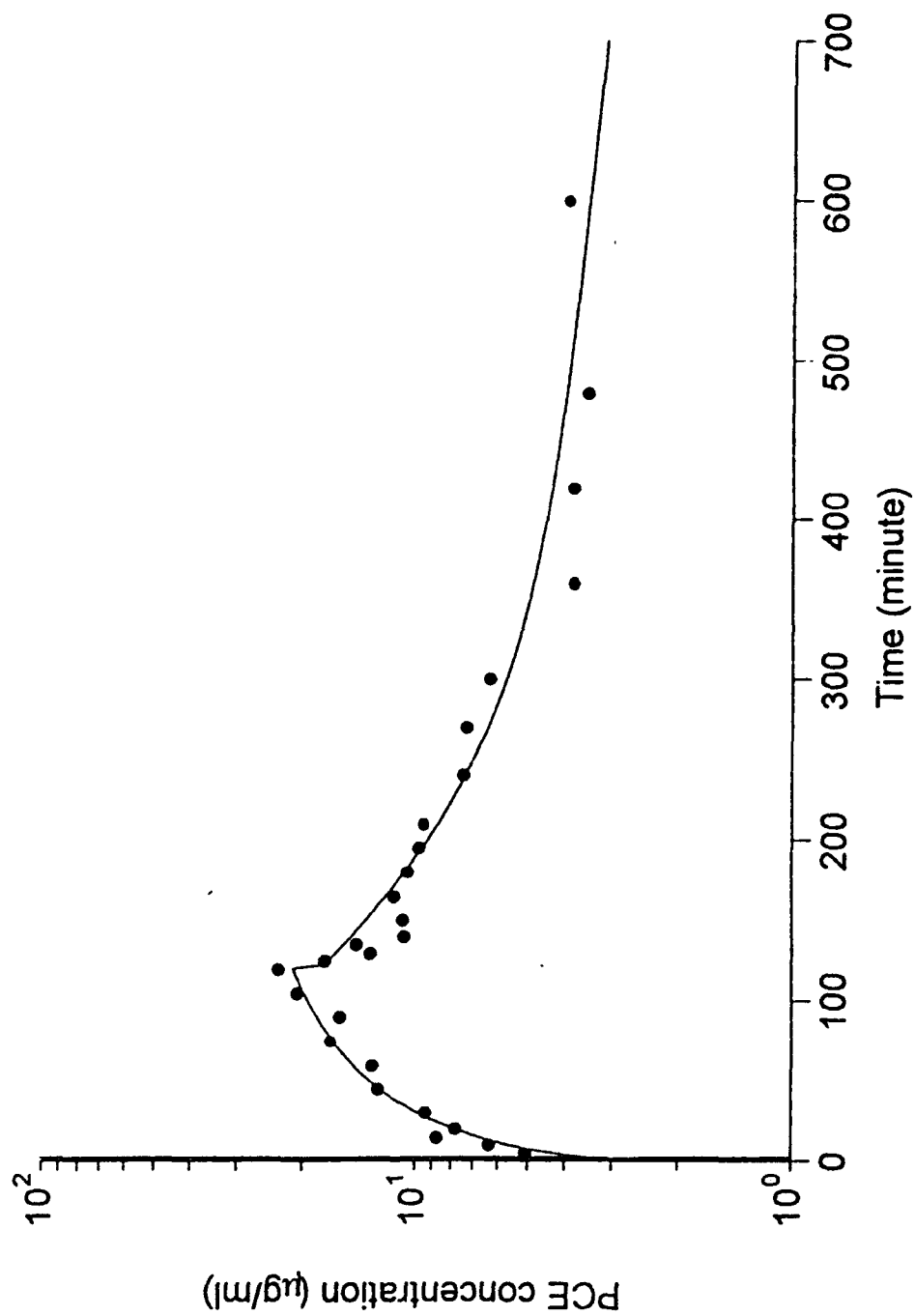
1. Diagram of the physiological pharmacokinetic model used to simulate the uptake and elimination of inhaled PCE. The symbols and parameters used to describe the model are included in Table 1.
2. Observed (•) and model-predicted (-) PCE concentrations in the arterial blood and exhaled breath of rats during and following a 2-hr, 500 ppm inhalation exposure. Each point represents the mean value for 6 rats.
3. Observed (•) and model-predicted (-) PCE concentrations in the arterial blood following a 2-hr, 50 ppm inhalation exposure. Each point represents the mean value for 6 rats.
4. Cumulative uptake of PCE during inhalation exposures to 500 (a) or 50 (b) ppm PCE for 2 hr. The quantity of inhaled PCE retained during successive 10-min intervals was calculated on the basis of the measured minute volume and difference between inhaled and exhaled PCE concentrations. Each point represents the mean \pm SE for 6 rats. Model predictions of cumulative uptake are delineated by the solid line.
5. Percent systemic uptake of PCE over time during inhalation exposures to 500 (a) or 50 (b) ppm PCE for 2 hr. Each point represents the mean \pm SE for 6 rats. Percent uptake of the inhaled dose was determined after 1, 3, 5, 10, 15 and 20 min and at 10-min intervals thereafter. Model predictions of percent uptake are delineated by the solid line.
6. Prediction of PCE exhaled breath concentrations using different lung:air partition coefficients. The influence of species differences in blood:air partition coefficients on PCE elimination in the exhaled breath was determined by comparison of predictions using different lung:air partition coefficients. The two lung:air partition coefficients that were employed were calculated using blood:air partition coefficients reported for the rat and the human,

according to the procedure detailed in the Results section. Differences in the exhaled breath PCE predictions using the two values are compared during and following 50 (a) and 500 (b) ppm exposures to PCE. All other model parameters are the same as in Table 1.

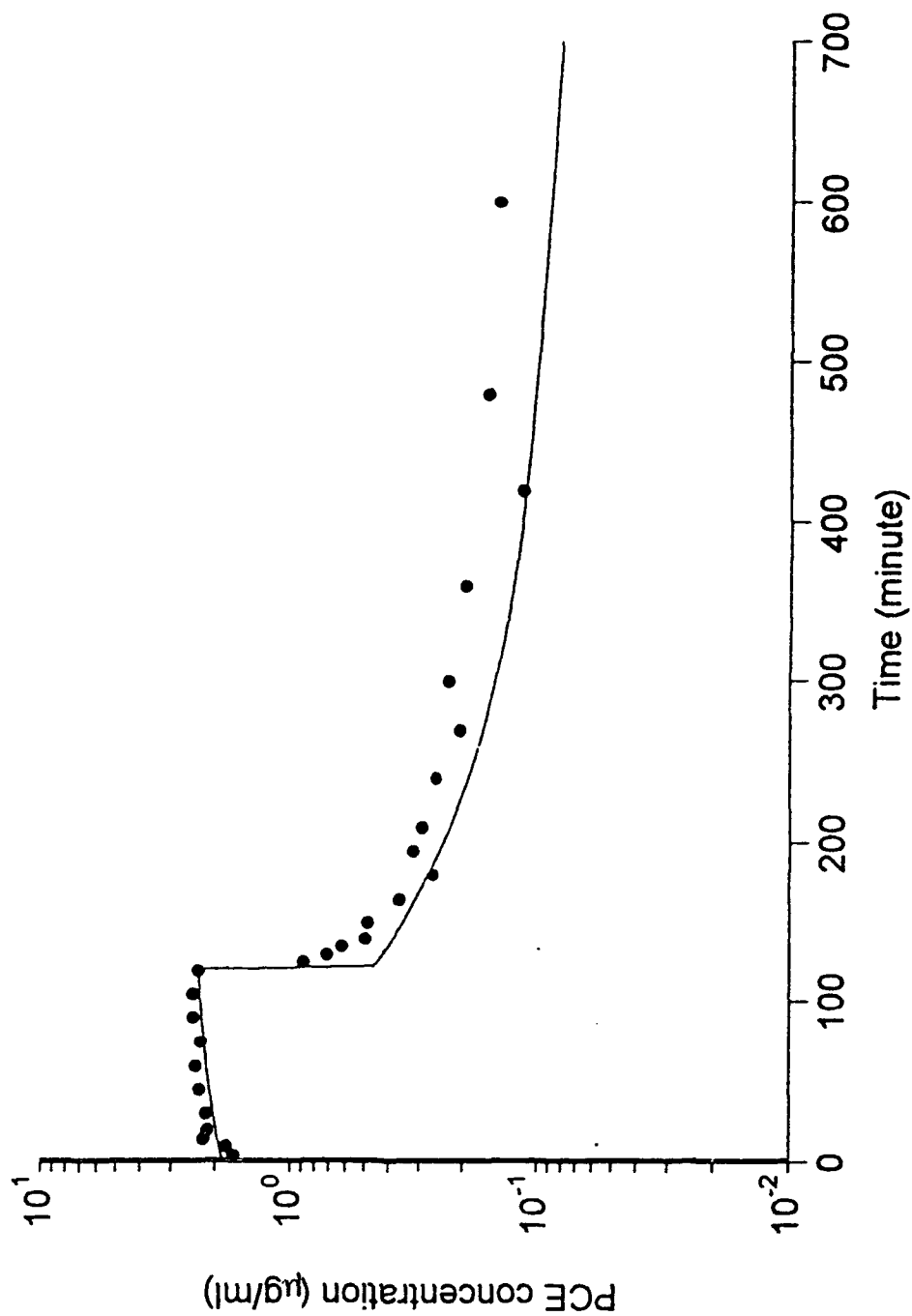
PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL OF PCE INTRAARTERIAL ADMINISTRATION



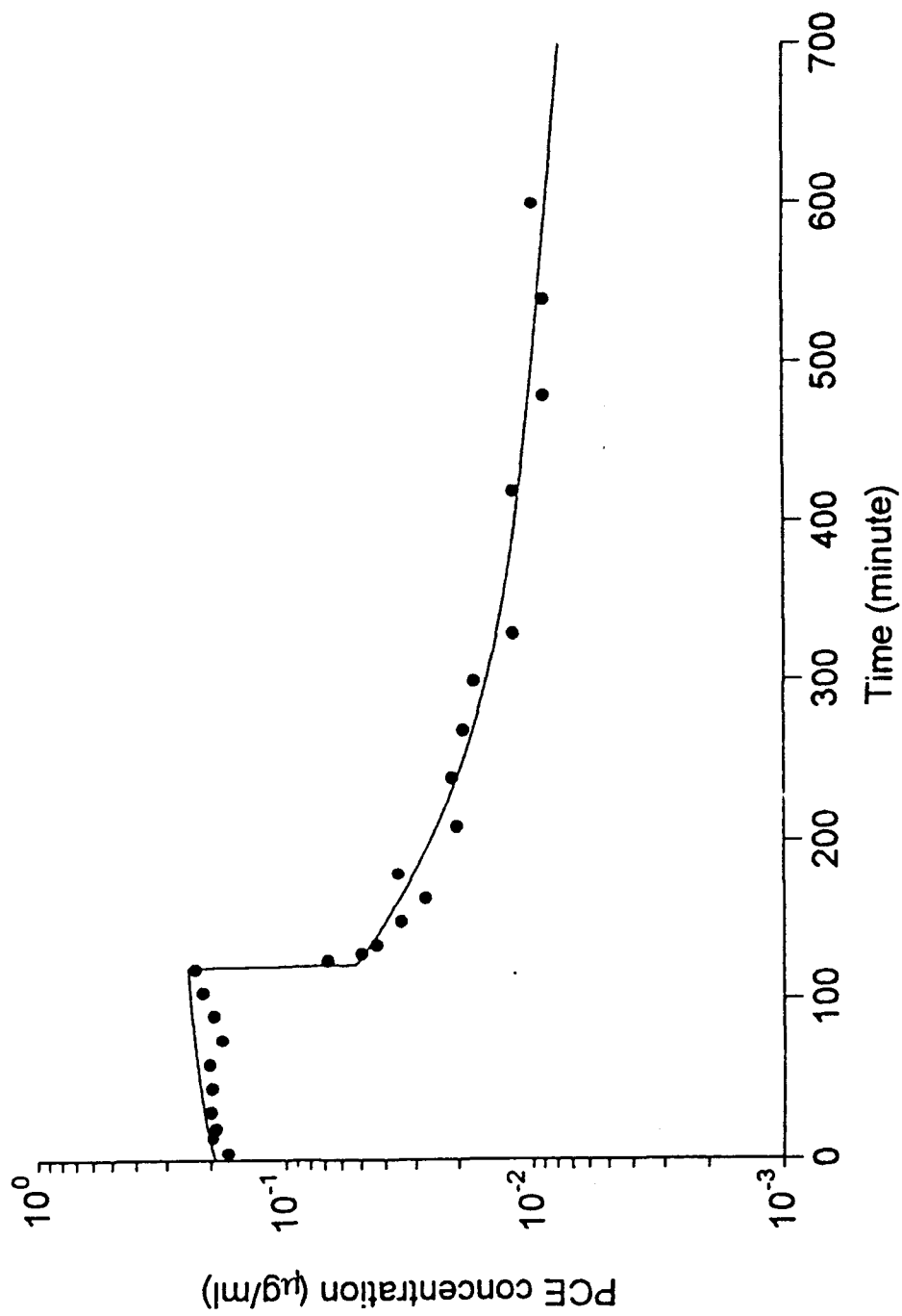
Arterial Blood (500 ppm)



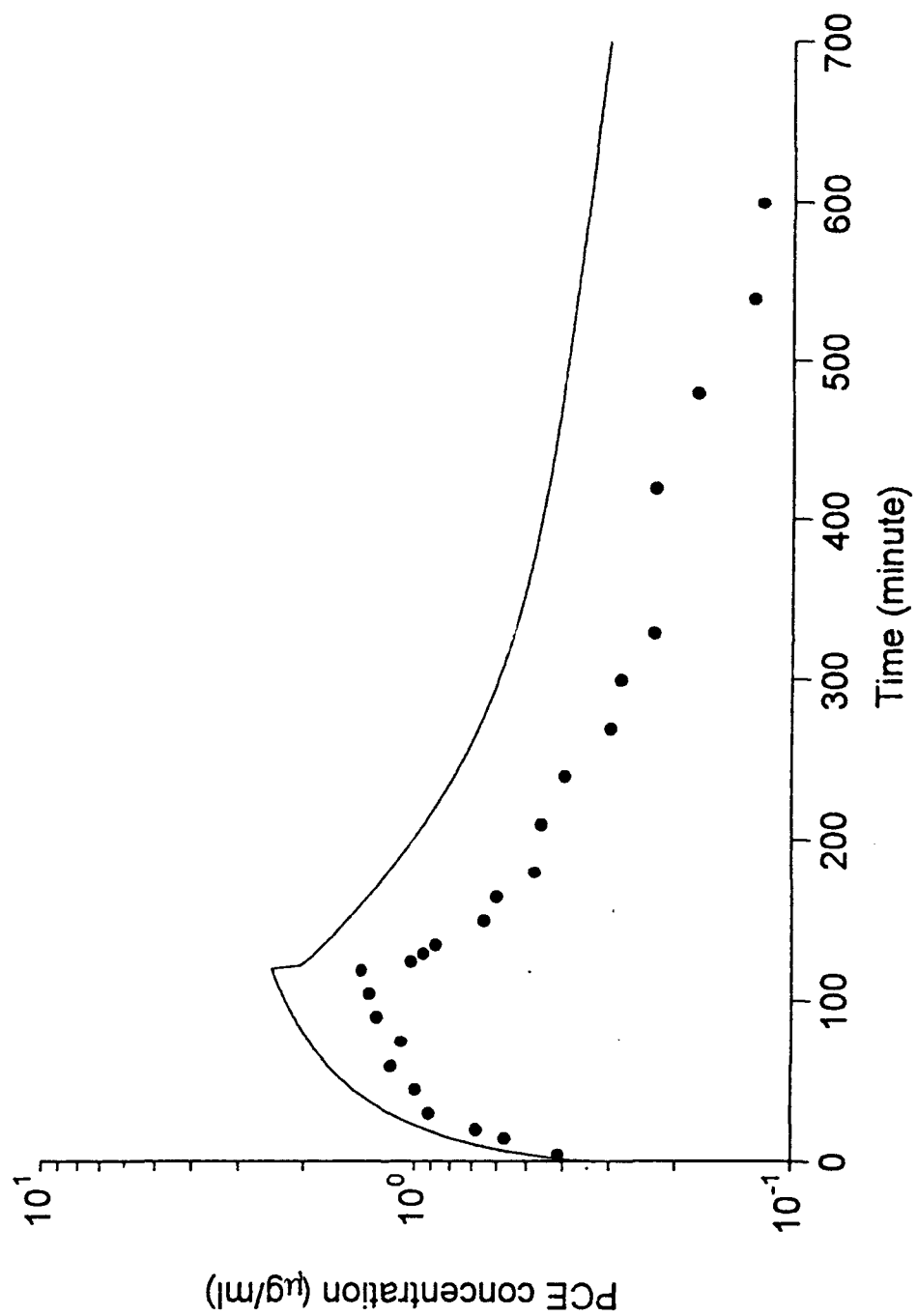
Exhaled Air (500 ppm)



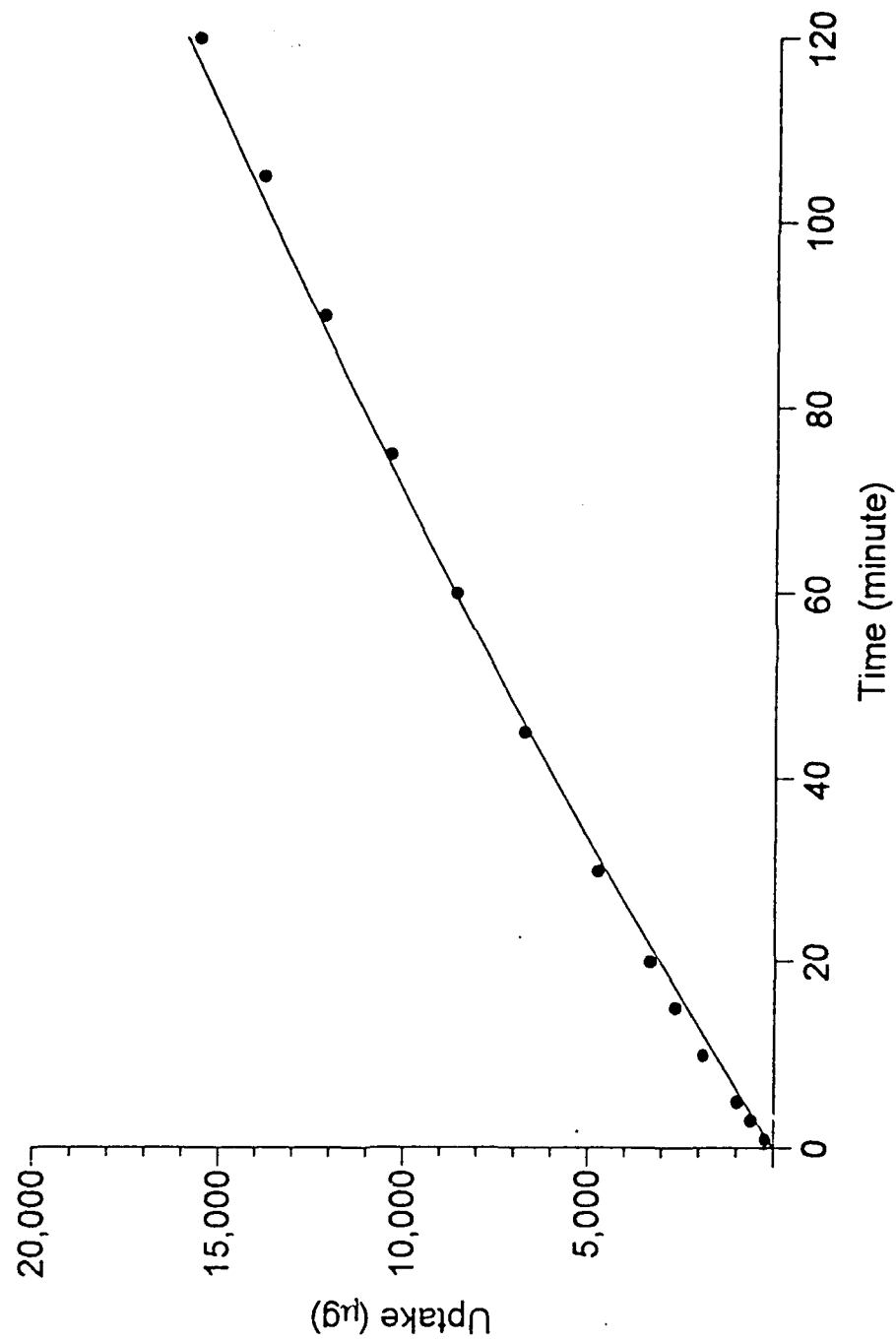
Exhaled Air (50 ppm)



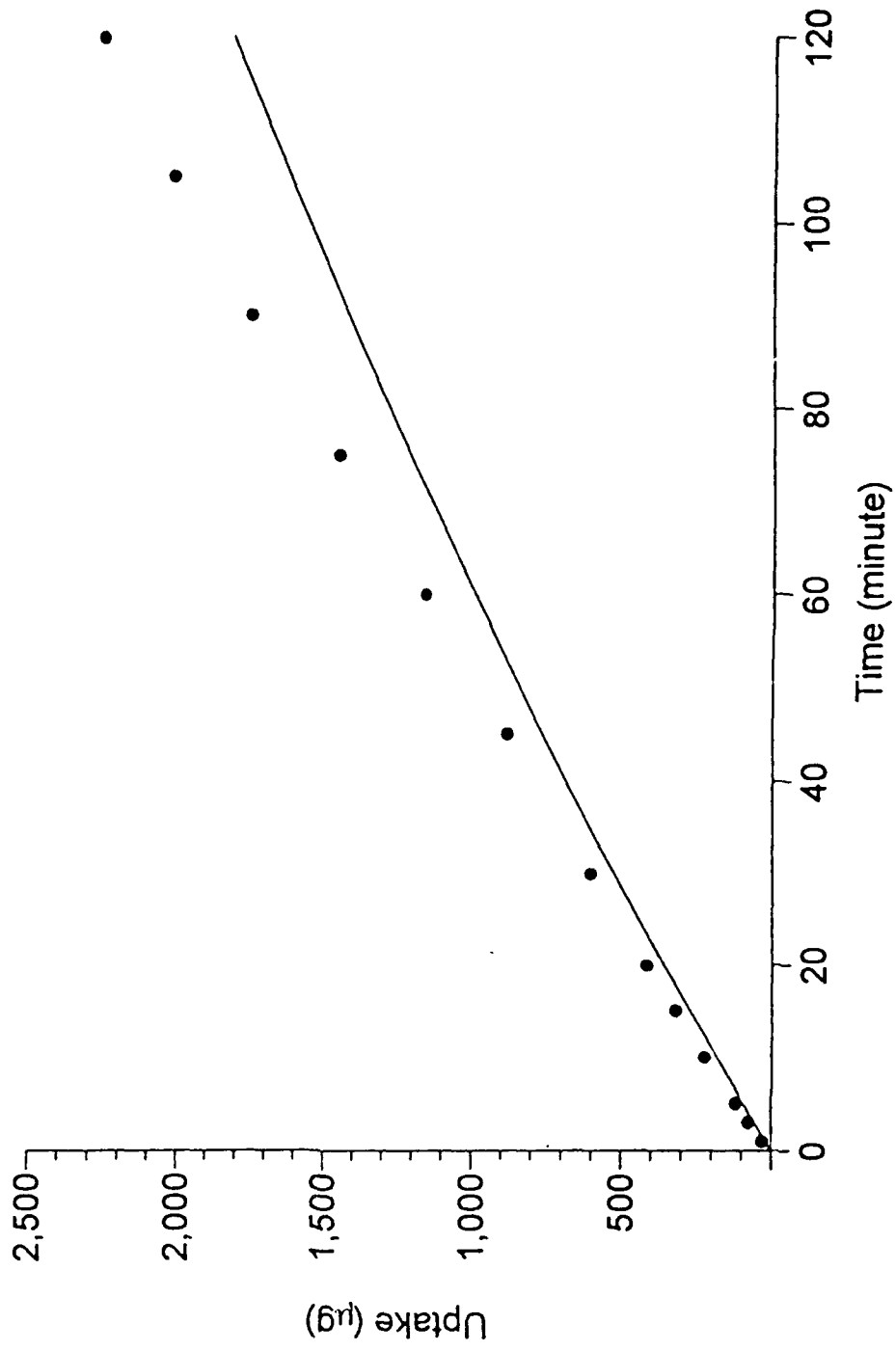
Arterial Blood (50 ppm)



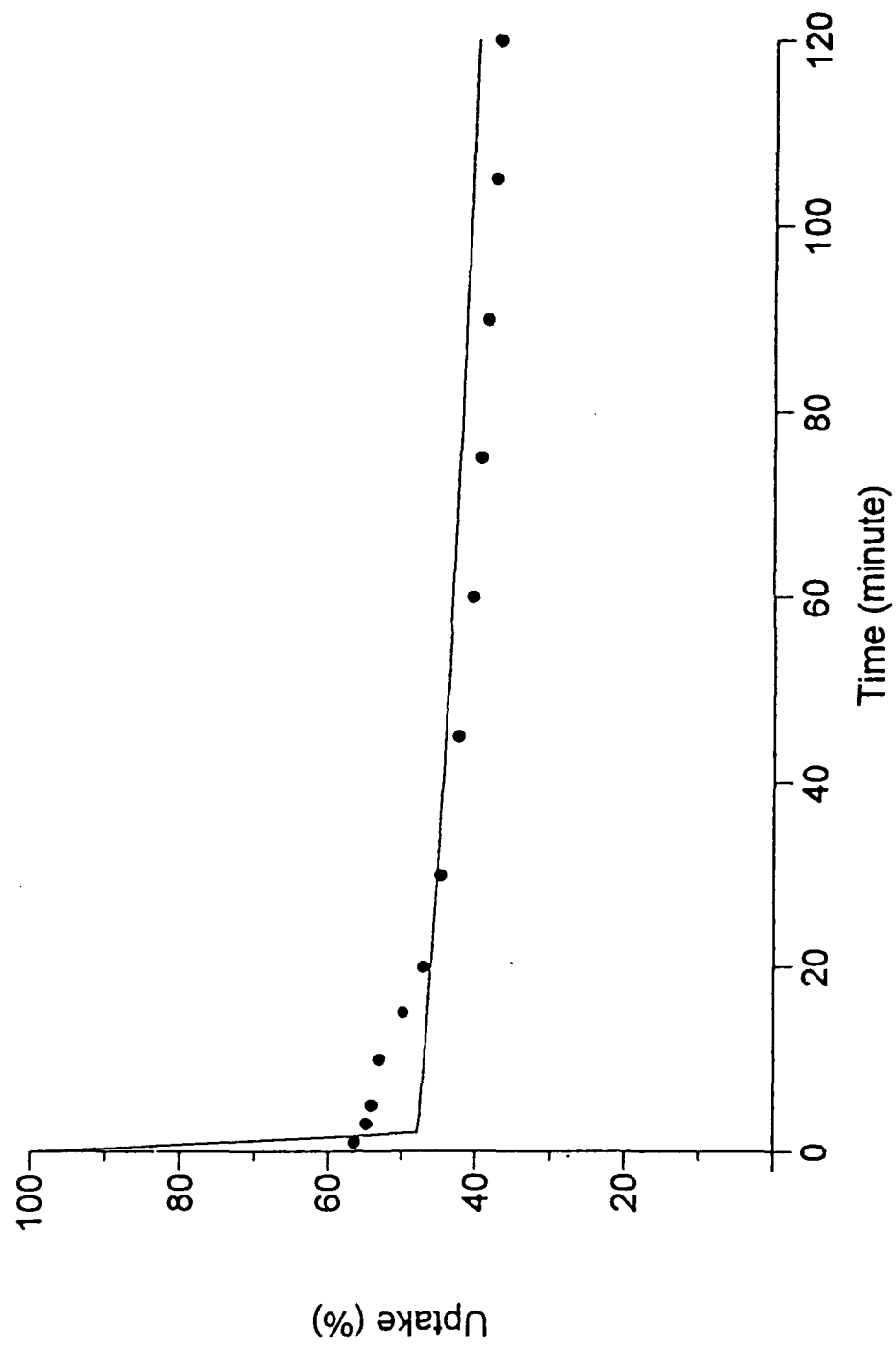
Cumulative uptake of PCE during inhalation of 500 ppm



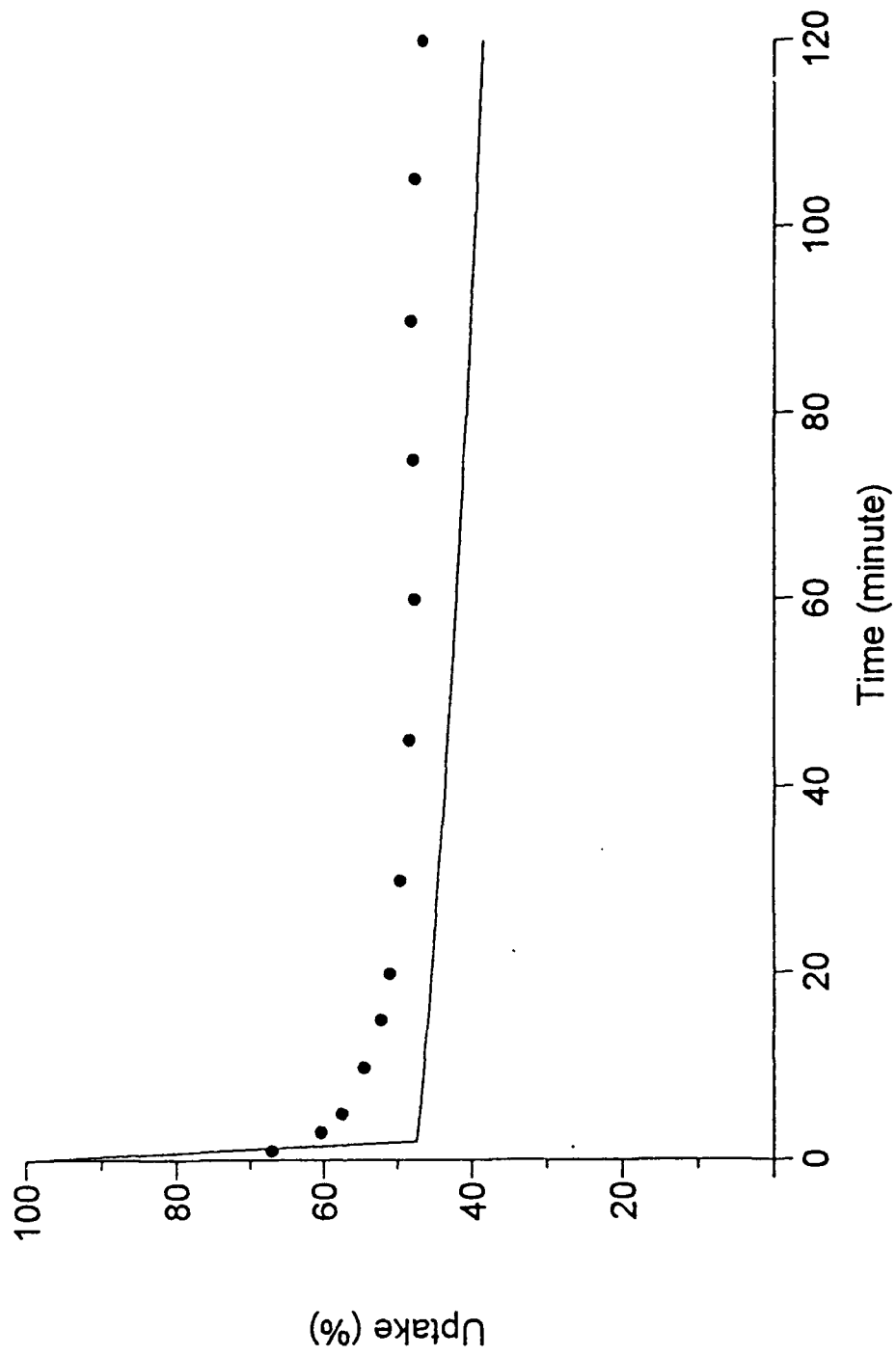
Cumulative uptake of PCE during inhalation of 50 ppm



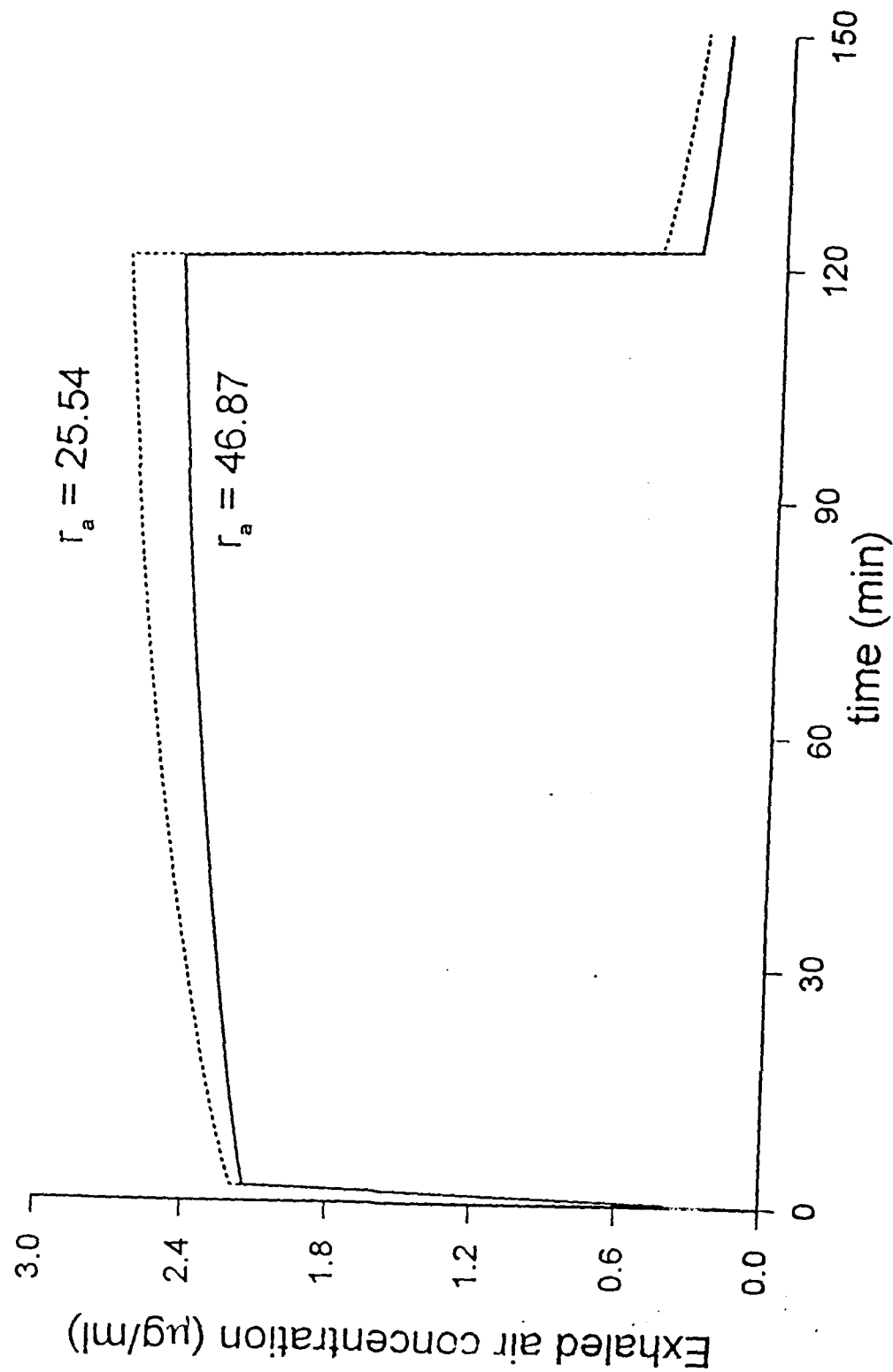
Percent uptake of PCE during inhalation of 500 ppm



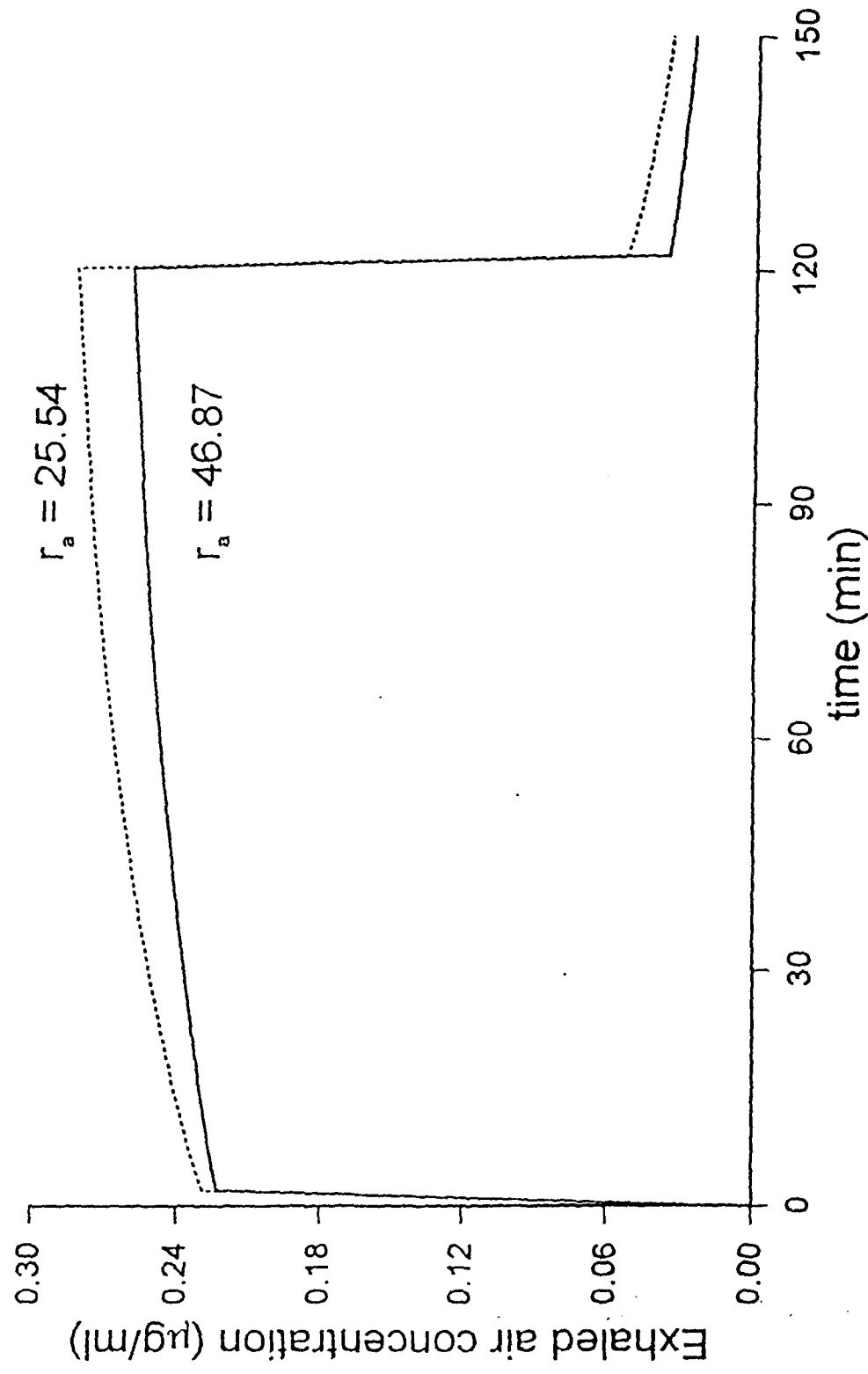
Percent uptake of PCE during inhalation of 50 ppm



Influence of the Lung:Air partition coefficients in exhaled air concentration (500 ppm)



Influence of the Lung:Air partition coefficient in exhaled air concentration (50 ppm)



APPENDIX

SECTION D

Manuscript to be submitted to
Fundamental and Applied Toxicology

Species Differences Between Rats and Dogs in
Physiologically-Based Model Parameter Estimation
Using Perchloroethylene Tissue Pharmacokinetics

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Abbreviated title: Physiological Model for Interspecies Tissue Doses

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ABSTRACT

Species Differences Between Rats and Dogs in Physiologically-Based Model Parameter Estimation Using Perchloroethylene Tissue Pharmacokinetics. DALLAS, C.E., CHEN, X.M., MURALIDHARA, S., VARKONYI, P., GALLO, J.M., TACKETT, R.L., AND BRUCKNER J.V. (1993) Fund. Appl. Toxicol. 0, 00-00.

The tissue disposition of perchloroethylene (PCE) was determined experimentally in two mammalian species of markedly different size, in order to obtain model input parameters from *in vivo* data for the development of a physiologically based pharmacokinetic (PBPK) model to forecast the disposition of PCE in each species. Male Sprague-Dawley rats and male beagle dogs received a single bolus of 10 mg PCE/kg bw in polyethylene glycol 400 by gavage. Serial samples of brain, liver, kidney, lung, heart, skeletal muscle, perirenal adipose tissue, and blood were taken for up to 48 hr following PCE administration. Blood and tissue PCE concentrations were analyzed using a gas chromatography headspace technique. The dogs exhibited considerably longer tissue and blood half-lives than did the rats. The larger of the two species had larger area under the tissue concentration (AUTC) versus time curves for all tissues except the liver. Whole body clearance of PCE in the rat was greater than in the dog. Model simulations indicated this was the result of more rapid and extensive PCE exhalation and metabolism by the rat. While the blood:air partition coefficient was similar to that value determined using *in vitro* data in the literature, tissue:blood partition coefficients were 1.4 to 2.8 times greater for the *in vivo* data in this study compared to the available published *in vitro* values. The PCE blood:air partition coefficient for the dog was twice that of the rat, and the tissue:blood partition coefficients were 1.5 to 3.0 times greater in the rat relative to the dog. These results demonstrate the importance of species

differences in partition coefficients for PBPK models for VOCs, and help to test important assumptions in interspecies scaling using the models.

Introduction

Physiologically-based pharmacokinetic (PBPK) models are being increasingly used in health risk assessments of chemicals, particularly in interspecies extrapolations of pharmacokinetic and toxicologic data. Similarities in the anatomy and physiology of mammalian species make scaling from one species to another possible, when physiological data are not available for a species of interest (Dedrick, 1973; Boxenbaum, 1984; Travis, 1987). By use of physiological and metabolic parameters which are appropriate for each species, accurate predictions of chemical concentrations in blood and tissues of different species are feasible with PBPK models.

Perchloroethylene (PCE) has been one of the most frequently used test chemicals in the ongoing development of PBPK models for use in different species. Utilizing a PBPK model for PCE with four tissue compartments, Ward et al. (1988) were able to adequately predict empirical data of other researchers for mice (Schumann et al., 1980; Buben and O'Flaherty, 1985), rats (Pegg et al., 1979), and humans (Stewart et al., 1961; Ikeda et al., 1972; Fernandez et al., 1976; Monster et al., 1979). The observed data consisted of PCE concentrations measured in exhaled breath over time and various metabolic parameters. These data were well simulated by the model of Ward et al. (1988) for each species. A similar approach was used by Travis (1987) for simulations of PCE kinetics in rats and humans, and by Chen and Blancato (1987) for mice, rats, and humans. There have been descriptions of the utility of PBPK models in predicting metabolite formation following PCE exposure of mice, rats, and humans (Bogen and McKone, 1988; Bois et al., 1990; Hattis et al., 1990). The focus of these metabolism studies was not to verify the accuracy of model predictions concentrations of PCE or its metabolites in target tissues, but to evaluate the

potential of using the models to simulate PCE metabolism for use in cancer risk assessments. PCE tissues concentrations were not employed in the development or validation of any these PBPK models.

In the development of these PBPK models, one of the most important model parameters is the tissue:blood partition coefficient. This value governs the rate of the transfer of the chemical between the blood and each of the tissue compartments represented by the model. In their PBPK model development for PCE, Ward et al. (1988), Bois et al. (1990), and Travis et al. (1987) have utilized partition coefficients derived from *in vitro* vial equilibration studies (Gargas et al., 1986 & 1989). An *in vivo* approach to deriving tissue:blood partition coefficients for PBPK models has been described (Gallo et al., 1987), in which the tissue-concentration time course of the test chemical is employed. While this approach provides an *in vivo* description of the rate of transfer of the chemical for each tissue for which the data is available, there has been little opportunity for its use because of the paucity of detailed tissue concentration time data for most VOCs, including PCE. Therefore, the time course of uptake, disposition, and elimination of PCE in blood and seven tissues was determined in two species, and the data utilized in deriving partition coefficients for a PBPK model for PCE. The rat and the dog were utilized in order to incorporate species of importance to toxicological and pharmacological testing, and to enable the evaluation of the utility of PBPK models to predict pharmacokinetics in species of wide variation in size using partition coefficients from detailed tissue concentration time data.

Methods

Male beagle dogs (6-15 kg) obtained from Marshall Farms (North Rose, NY), and male Sprague-Dawley rats (325-375 g) obtained from Charles River Laboratories (Raleigh, NC), were employed in these studies.

The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. Rats were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow® were provided *ad libitum*. The rats were used after at least a 7-day acclimation period, at which time they were approximately 12 weeks old. Dogs were housed in dog runs and also given a 14-day acclimation period, and were fed Purina Dog Chow®. Dogs were used between 6-9 months of age and in a weight range of 6-15 kg. Solvent exposures were initiated between 1000 and 1200 hr each day.

1,1,2,2-Perchloroethylene (PCE) (tetrachloroethylene) of 99%+ purity was obtained from Aldrich Chemical Co. (Milwaukee, WI). The purity of the chemical during the conduct of the study was verified by gas chromatography. For intraarterial (ia) administrations to rats, a cannula was surgically implanted in the common carotid artery. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml):acepromazine maleate (10 mg/ml):xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v/v/v). The cannulated animals were allowed relative freedom of movement during a 24-hr recovery period with the cannulas protected from manipulation by surgical tape at the back of the head. All animals employed in the tissue kinetic studies were unanesthetized. PCE was administered as a single bolus po at a dose of 10 mg/kg, using polyethylene glycol 400 volume/kg bw as a vehicle.

Groups of 3 dogs each were serially sacrificed (using etherization) at the following times post dosing: 1, 5, 10, 15, 30 and 60 min, and 1, 2, 4, 6, 12, 36, 48 and 72 hr. Blood samples were obtained by cardiac puncture. Approximately 1 g samples of liver, kidney, brain, lung, heart, perirenal fat, and skeletal muscle were then quickly removed and placed into previously chilled 20-ml glass vials containing 2 ml of ice-cold saline and 8 ml of isooctane. Each tissue was then homogenized for an established time interval with a Ultra-Turrax SDT homogenizer (Tekmar Co., Cincinnati, OH). These times were kept as brief as possible in order to minimize volatilization of the halocarbons. Brain, liver, and fat were the most easily homogenized, requiring only 3-4 sec. Kidney, lung and heart required 5-8 sec. Skeletal muscle was the most difficult to homogenize, in that it required 20 sec. The homogenates were then centrifuged at 1800 x g for 10 min at 4°C in capped vials. An aliquot of the isooctane layer was transferred to 8-ml headspace vials. These vials were capped immediately with rubber septa and a spring washer and crimped to insure an airtight seal. Each sample vial was then placed into the autosampler unit of a gas chromatograph (GC). The limit of detection for PCE was approximately 1 ng, or 6.7 parts of PCE per billion parts of air in the sample vial.

A model Sigma 300 GC equipped with a HS-6 head space sampler (Perkin Elmer Co., Norwalk, CT) was used for the analysis of the halocarbon. The GC was equipped with an electron capture detector. Analyses were carried out on stainless-steel columns (182 cm x 0.317 cm) packed with 10% FFAP (Alltech Associates, Deerfield, IL). The GC operating conditions were: headspace sampler temperature, 90°C; injection port temperature, 200°C; column temperature, 110°C; detector temperature, 400°C; flow rate for argon/methane carrier gas, 60 ml/min. All analyses were conducted using a 20- μ l aliquot of isooctane in the 8-ml

headspace vials. Each sample vial was heated thermostatically for 10 min in the GC autosampler unit, pressurized for 30 sec with the carrier gas, and injected automatically by a solenoid-controlled mechanism into the GC column. These conditions resulted in vaporization of the halocarbons in the sample vials, since PCE was heated to a temperature slightly below its boiling point, and the vial subsequently pressurized and vented into the GC.

The area under the concentration-time curve (AUC) for blood and tissues was determined from the time of administration to infinity. Total body clearance was determined as the dose divided by the blood AUC in each species. Bioavailability was calculated by AUC_{po}/AUC_{ia} . The maximum concentration reached in blood and tissues (C_{max}) and the time after dosing that it occurred (T_{max}) were determined by observation of the available data points.

Metabolism and exhalation of PCE in the rat and the dog were predicted using a physiologically-based pharmacokinetic (PBPK) model for ia administration in the rat and po administration in the dog (Fig. 1). It was similar to PBPK models previously developed by Angelo and Pritchard (1984) and Ramsey and Andersen (1984) for other VOCs, in that it provided for inhalation and exhalation through a lung compartment, metabolism in a liver compartment, and blood-flow-limited organ representations. The current model differed primarily in that it included a greater number of tissue compartments. Values determined in this laboratory were employed for tissue volumes and blood flows (Delp et al., 1991) and alveolar ventilation (Dallas et al., 1991) for the male Sprague-Dawley rat. *In vivo* tissue:blood partition coefficients were calculated from ia data for the rat (Dallas et al., 1993) and oral data for the dog obtained in the present study by the area method of Gallo et al., (1987). The metabolic parameters K_m and V_{max} , and blood:air coefficients were estimated from the observed data by nonlinear

regression analysis. Parameters bioavailability (f) and the absorption rate constant (K_a) were estimated from oral data by fitting of the blood and liver concentration versus time data for both species. Tissue volumes and blood flows for the dog were obtained from the literature. Sources of tissue volumes were as follows: liver, heart, lung, muscle, and blood (Andersen 1970), kidney (Spector, 1956), and fat (Sheng and Huggins, 1971). Sources of blood flows were as follows: liver (Liang et al., 1982), kidney, muscle, and brain (Humphrey and Zins, 1983), cardiac output (Andersen, 1970), fat (Nagasaka et al., 1976), and heart (Liard et al., 1982). Values for alveolar ventilation in the dog were taken from the publication by Andersen (1970).

Differential mass balance equations, incorporating the parameters listed in Table 1, that described the transport of PCE in the rat and the dog were numerically integrated with the Advanced Continuous Simulation Language (ACSL) computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted PCE concentrations over time. The model-predicted cumulative uptake values were equal to the sum of the simulated amounts of PCE in each tissue compartment in the model.

Results

Pharmacokinetic parameters for PCE in the rat for po administration are presented in Table 2. Maximum tissue concentrations (C_{max}) were achieved rapidly following po administration. After oral dosing the C_{max} was reached in 10 to 15 min for each non-lipoidal tissue, other than lung and muscle, which required 60 min. Uptake into the adipose tissue was quite slow, in that its C_{max} was 360 min. As would be anticipated for a chemical as highly lipophilic as PCE, the C_{max} , AUC and $t_{1/2}$ values for the adipose tissue were substantially greater than

for all other tissues following po administration. The $t_{1/2}$ for nonlipid tissues was relatively similar in rats, with liver and muscle having the shortest half-lives. The C_{max} and AUC attained in the liver was considerably higher than in the other nonlipid tissues.

The degree of blood perfusion had a significant impact on tissue disposition of PCE in the rat. Highly perfused organs such as kidney and brain had similar C_{max} , AUC, and $t_{1/2}$ values for each route of administration. Poorly perfused and nonlipoidal tissues, such as skeletal muscle, had lower C_{max} and AUC values than these highly perfused tissues.

Pharmacokinetic parameter estimates following oral administration of PCE to the dog are presented in Table 3. The T_{max} for all nonlipoidal dog tissues was 60 min., which was 4-6 times longer than for most of the tissues in the rat. The T_{max} for dog fat was also twice as long as for the rat, though the C_{max} in the two species was nearly equivalent. As in the rat, much greater deposition of PCE was observed in the fat than in the other tissues. Except for the brain, C_{max} and AUC values for the fat were at least 8 and 33 times higher, respectively, than in nonfat tissues. PCE accumulation in the dog brain was considerably higher than in the other nonfat tissues. The rat also exhibited relatively high brain C_{max} and AUC values, though they were even higher in the liver following oral administration (Table 3). The elimination $t_{1/2}$ for PCE from the dog brain was considerably longer than from other tissues, whereas the $t_{1/2}$ in rat brain was not different from most other tissues. There was a difference in PCE deposition between highly perfused tissues (e.g., brain) and poorly perfused and nonlipoidal tissues (e.g., muscle) in the dog and the rat. AUCs for dog tissues were greater than for corresponding rat tissues following oral administration of PCE, except for liver. The greater AUCs in dog tissues can be

attributed largely to prolonged elimination. The $t_{1/2}$ values in the nonfat dog tissues were 4-12 times greater than in corresponding rat tissues. Following oral administration, whole body clearance of PCE was 26.9 and 4.3 ml.min/kg for rats and dogs, respectively.

The time-course of exhalation and metabolism of PCE were predicted by the model for the rat (Fig. 8) and dog (Fig. 9) following ia and po administration, respectively, of the chemical. For each species, the fraction of PCE exhaled was substantially greater than the fraction metabolized. The rate and magnitude of exhalation and metabolism were higher in the rat than in the dog.

DISCUSSION

Tissue concentration-time data were used in this study in an *in vivo* approach to deriving model input parameters to assist in the development of a PBPK model for PCE. Previously, such detailed tissue concentration-time data sets have not been available for most VOCs, including PCE. It has been proposed that the most logical measure of target organ exposure (and therefore toxic injury) is the area under the tissue concentration versus time curve (AUTC) (Andersen, 1987). The AUTCs measured in the present study, following oral administration of PCE to rats and dogs, have proven useful in helping verify some previous assumptions concerning the interspecies scaling of tissue disposition. Interspecies scaling has been described as the determination of how target tissue exposure is affected by the size of a species for a particular administered dose (Andersen, 1987). In interspecies scaling of tissue doses using PBPK models, the National Research Council has made several important assumptions (NRC, 1986). Scaling metabolic and physiological clearance as a power of body weight and tissue volumes in proportion to body weight, it was predicted that a greater AUTC

for parent compounds would be obtained in larger animals than in smaller animals. The observed PCE tissue concentrations in two dissimilar species in the current study are in agreement with this. AUTCs were significantly greater for six tissues in dogs than for the same tissues in rats following an equivalent oral dose of PCE. Andersen (1987) concluded that clearance would be expected to be greater in smaller species, because it increases as a fractional power of body weight, and that this would account for lower AUTC values in the smaller of two animal species. Indeed, clearance of PCE in the present investigation was significantly greater in rats than in dogs following an equal dose of PCE. The major route of PCE elimination is exhalation (Schumann et al., 1980), which is governed largely by the blood:air partition coefficient. This parameter is smaller in rats (19.6) than in dogs (40.5), so there will be a higher rate of exhalation of PCE by the rat at equal blood levels. However, blood levels are influenced to some degree by most model input parameters, so the difference in blood:air partition coefficient alone is not sufficient to determine which species will have the higher exhalation rate. It should be noted that both exhalation and metabolism were predicted to be higher in the rat than in the dog by the PBPK model in the current investigation.

The primary approach that has been employed for deriving partition coefficients for VOCs to use in PBPK models has been the vial equilibration technique by Gargas et al. (1989). This method was modified from an earlier approach using a headspace vial equilibration technique for blood, oil, and water (Sato & Nakajima, 1979) and another approach using tissue homogenates (Fiserova-Bergerova et al., 1984). A similar blood:air partition coefficient for PCE was reported using this *in vitro* approach (18.9) as was observed using the *in vivo* data in the current study (19.6). The tissue:blood partition coefficients were

consistently higher using the *in vivo* approach relative to the *in vitro* technique. The coefficients for which comparisons are possible, liver, fat, and muscle, were 1.4, 1.8, and 2.8 times higher, respectively, in the current study compared to the values using the vial equilibration technique (Gargas et al., 1989). Differences between species for the PCE blood:air partition coefficient using the *in vitro* approach were minimal between mice and rats, but the value for both these species were more than 75% greater than that for humans (Gargas et al., 1989). In the current study, the blood:air coefficient of the dog was more than twice that of the rat. All of the PCE tissue:blood partition coefficients were between 1.5 and 3.0 times greater in the rat than in the dog. These results demonstrate the importance of species differences in partition coefficients in developing PBPK models for VOCs in interspecies extrapolations.

In an evaluation of the uncertainties involved in PBPK models using PCE as an example, Hattis et al. (1990) determined that there were appreciable differences between the model predictions. Of the many potential sources of uncertainty, it was concluded that the primary cause of differences in model predictions of risk were the approaches employed in estimating the metabolic parameters. There were 20- and 60-fold differences in estimates of K_m for the various mouse and rat models, respectively. V_{max} estimates differed by 8- and 15-fold for the mouse and rat models, respectively. The values for K_m and V_{max} in the present study were estimated by nonlinear regression, fitting the model predictions to the observed blood and liver concentration values of PCE. Expressing V_{max} in the same units as in Hattis et al. (1990), our V_{max} value (2.9 nM/ml Kg) for rats is 10 times smaller than the range of values reported there. Of course, the researchers who made these larger estimates of V_{max} did not have liver concentration-time data available for determination of this parameter. Due

to the dependence of PBPK models on a number of variables, there are of course other potential sources of error such as model structure and input parameters.

Farrar et al. (1989) concluded that the structural uncertainty (e.g., number and selection of compartments, accounting for elimination) involved in the use of various dose metrics, such as AUTC and AUC (for arterial blood) in interspecies extrapolations, was of greater importance than the uncertainty associated with the values of PBPK model input parameters such as partition coefficients. Using the *in vitro* partition coefficients of Gargas et al. (1989), the precision of PBPK model predictions of the rate of metabolite formation following PCE exposure has been reported to be within 22%, on average, from the observations available for rats, mice, and humans (Bois et al., 1990). Despite the stated uncertainties in the model parameters, Bogen and McKone (1988) concluded that their steady-state PBPK model for PCE was capable of yielding appropriate estimates of total metabolized PCE using time-weighted air concentrations in inhalation exposures.

While the detailed tissue concentration time data from the current study provides useful information as an *in vivo* method for PBPK model parameter estimation, the approach is time-consuming and expensive. The *in vitro* vial equilibration technique does not require extensive laboratory equipment or measurement of the chemical in the test medium (Gargas et al., 1989), and would certainly be the method of choice for most partition coefficient estimation needs for PBPK model parameter estimation for VOCs. Where available, *in vivo* tissue concentration time data can help to evaluate *in vitro* data, contribute to the design of tissue compartments of PBPK models, and test important assumptions in interspecies scaling of the models.

Table 1
Parameters Used in the Physiologically Based
Pharmacokinetic Model for PCE in the Rat and the Dog

Parameter	Rat	Dog
Body Weight (kg)		
Tissue volumes (ml)	Percentage of Body Weight	
liver	3.39	4.22
kidney	0.77	0.6
fat	5.0	15.2
heart	0.33	1.13
lung	0.37	0.66
muscle	35.36	46.8
brain	0.6	0.75
blood	7.40	8.19
rest of body	46.78	22.45
Alveolar ventilation (ml/min)	1.54 (ml/min·g) BW(g) ^{0.75}	2.576 (ml/min·g) BW(g) ^{0.75}
Cardiac output	1.57 (ml/min·g) BW(g) ^{0.75}	1.05 (ml/min·g) BW(g) ^{0.75}
Blood flows (ml/min)	Percentage of Cardiac Output	
liver	15.73	5.12
kidney	13.13	10.1
fat	6.56	5.02
heart	4.73	3.37
lung	100	100
muscle	26.11	40.59
brain	2.21	3.12
blood	100%=1.57 (ml/min·g) BW(g) ^{0.75}	100%=2.05 (ml/min·g) BW(g) ^{0.75}
rest of body	31.53	32.68
Partition Coefficients		
Blood:air	19.6	40.5
Fat:blood	152.5	63.2
Lung:blood	2.47	0.97
Liver:blood	5.25	1.64
Muscle:blood	2.98	1.9
Brain:blood	4.37	2.66
Heart:blood	2.68	1.74
Kidney:blood	4.45	1.49
Rest of body:blood	2.98	1.9
Metabolism constants		
V _{max} (μg/min)	0.15	0.85
K _m (μg/ml)	0.019	0.023
f	0.53	0.68
k _a	0.025	0.34

Table 2
Pharmacokinetic Parameters in the Rat Following
Oral Administration of 10 mg PCE/kg bw¹

Tissue	Area under curve ($\mu\text{g}\cdot\text{min}/\text{ml}$)	Biological half-life (min)	Cmax ($\mu\text{g}/\text{g}$)	Tmax (min)	Bioavailability (F)
Liver	1717	278	12.30	10	0.99
Kidney	1112	328	5.45	10	0.63
Fat	47815	573	41.42	360	0.79
Heart	820	327	2.85	15	0.77
Lung	637	302	2.0	60	0.70
Muscle	970	271	2.12	60	0.82
Brain	1407	307	5.05	15	0.81
Blood	333	349	0.99	15	0.84

¹Each value represents the mean for 4 rats and 14 time points ranging from 1 min to 72 hr.

Table 3
Pharmacokinetic Parameters in the Dog Following
Oral Administration of 10 mg PCE/kg bw¹

Tissue	Area under curve ($\mu\text{g}\cdot\text{min}/\text{ml}$)	Biological half-life (min)	Cmax ($\mu\text{g}/\text{g}$)	Tmax (min)
Liver	1564	2124	5.31	60
Kidney	1431	1281	4.18	60
Fat	60809	949	42.75	720
Heart	1672	1813	5.40	60
Lung	937	1615	2.26	60
Muscle	1824	1547	3.00	60
Brain	2563	3682	9.65	60
Blood	962	1121	1.27	60

¹Each value represents the mean for 3 dogs and 6 time points ranging from 1 to 72 hr.

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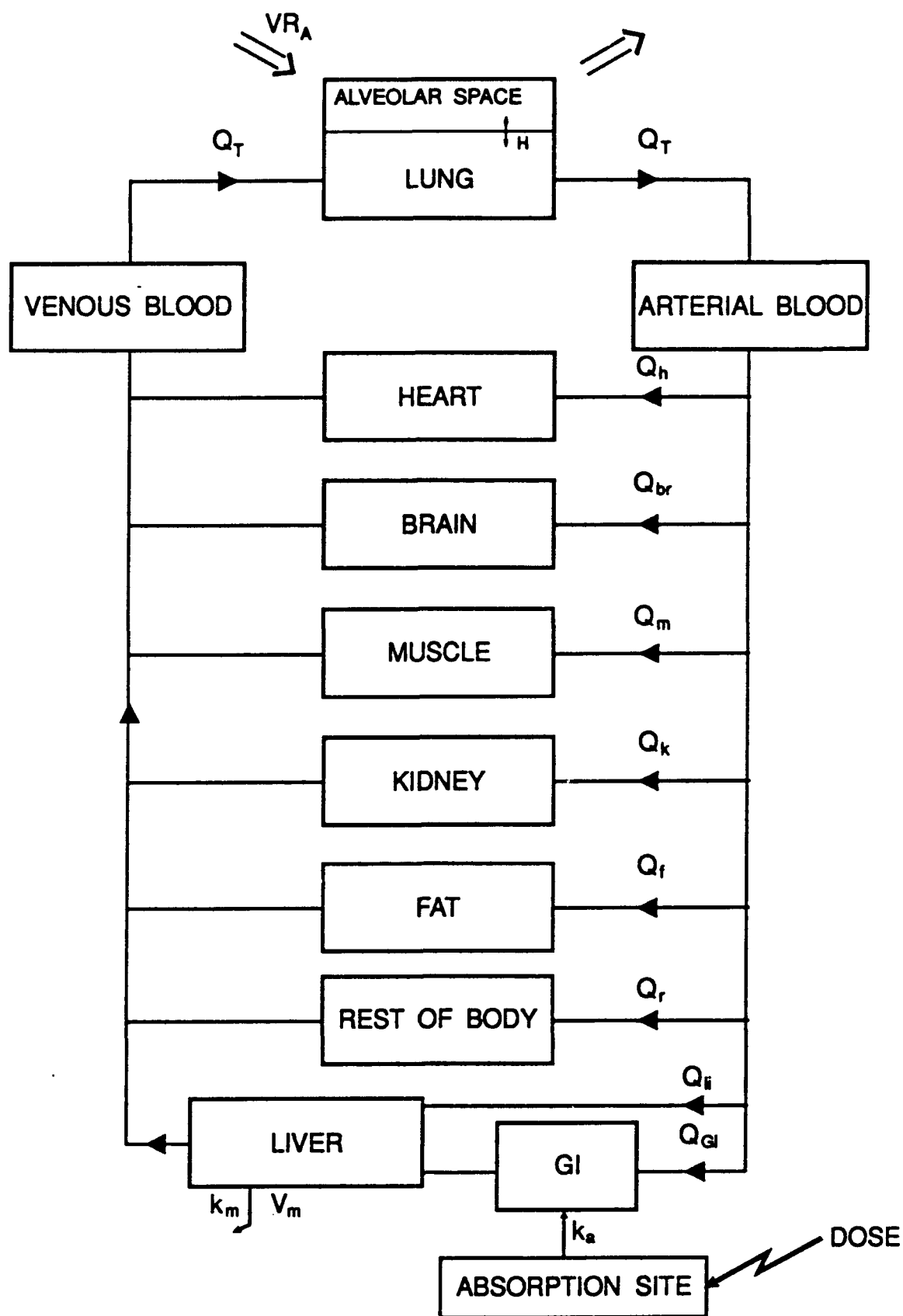
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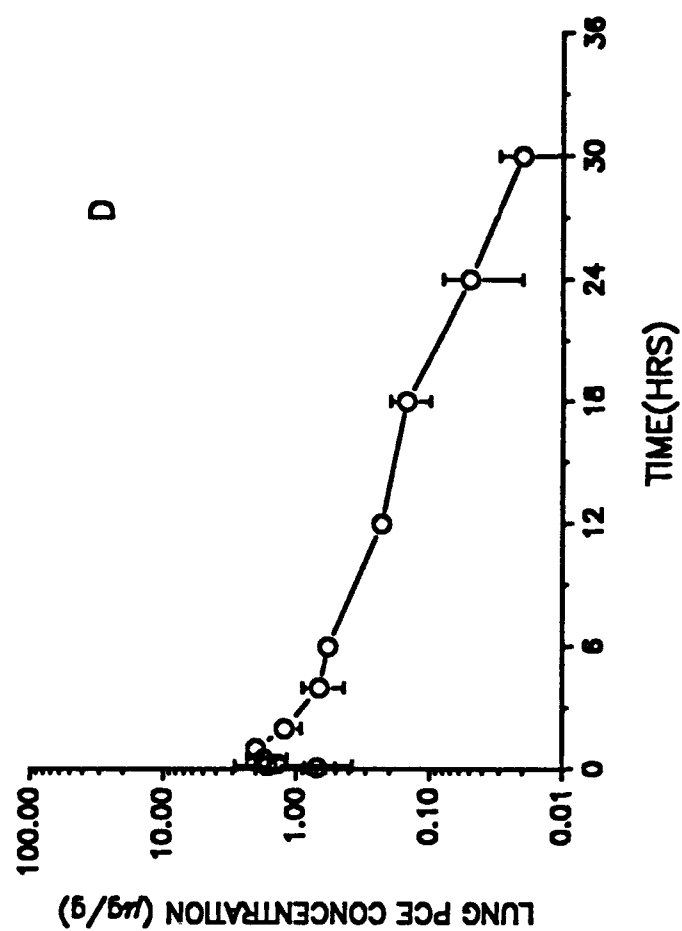
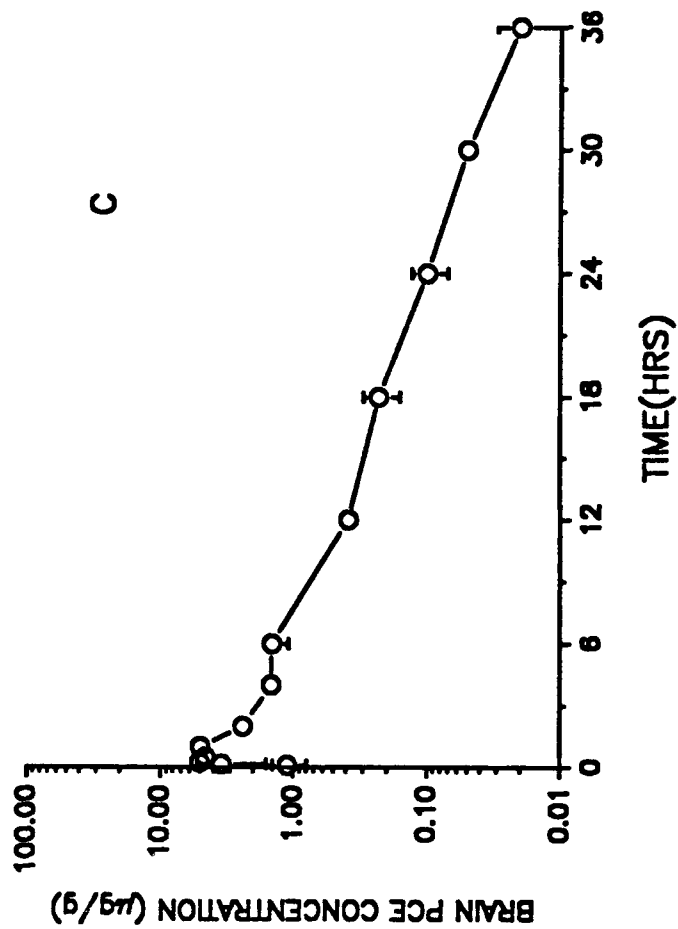
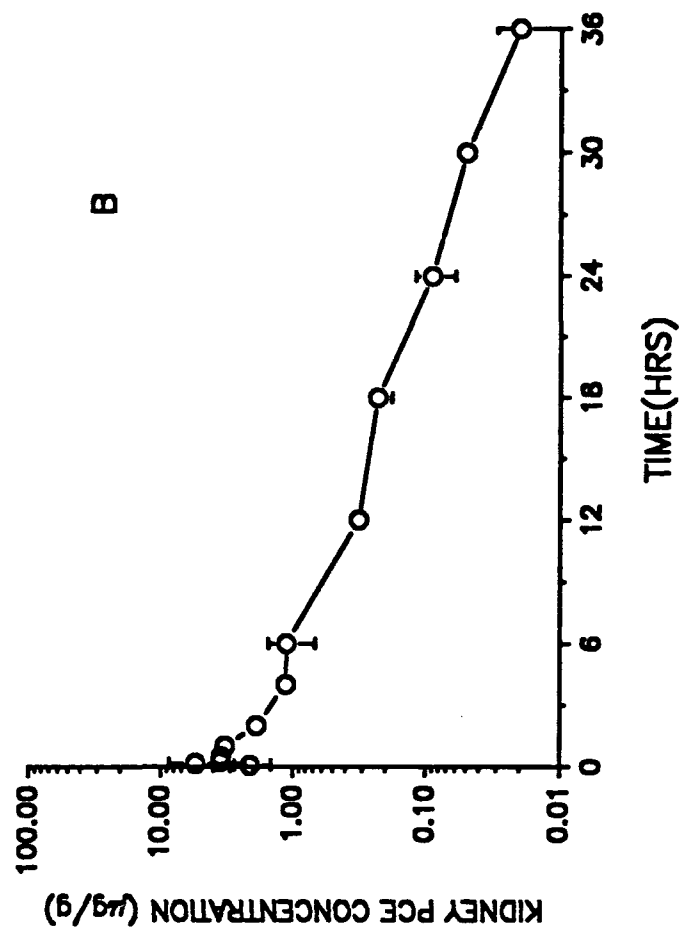
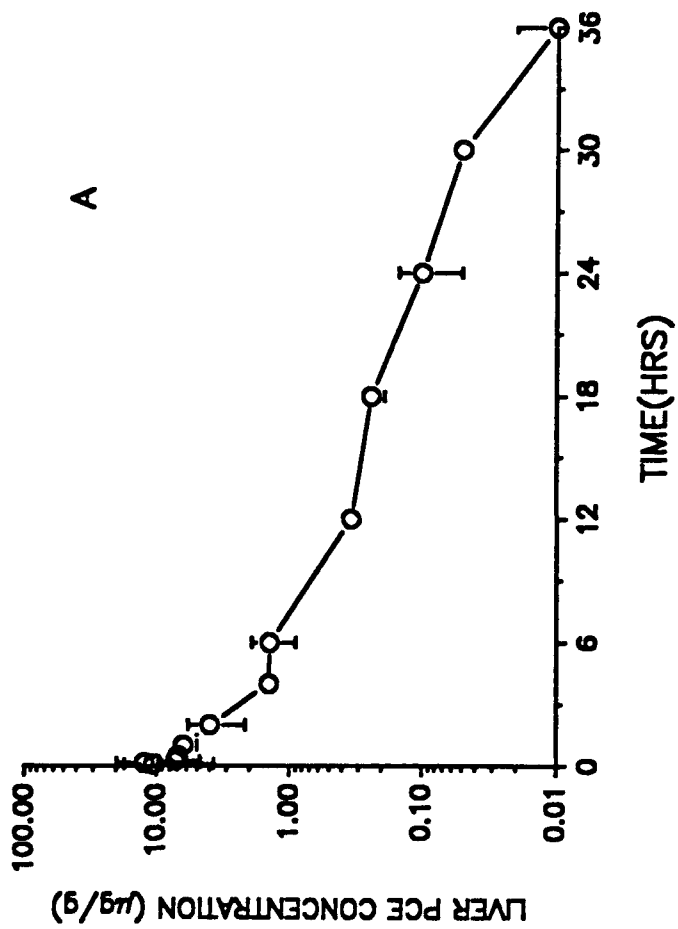
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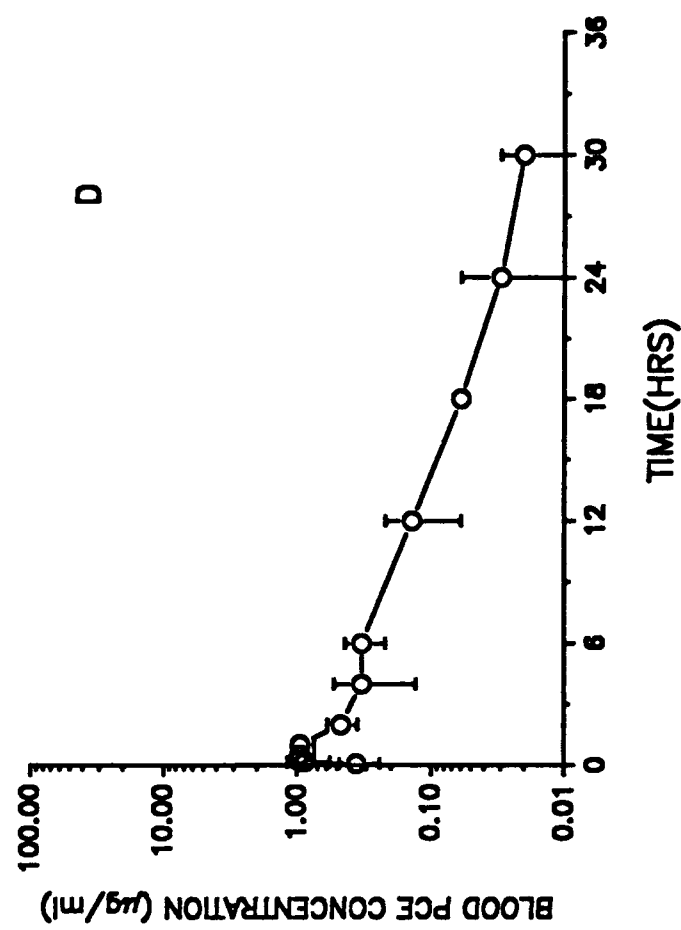
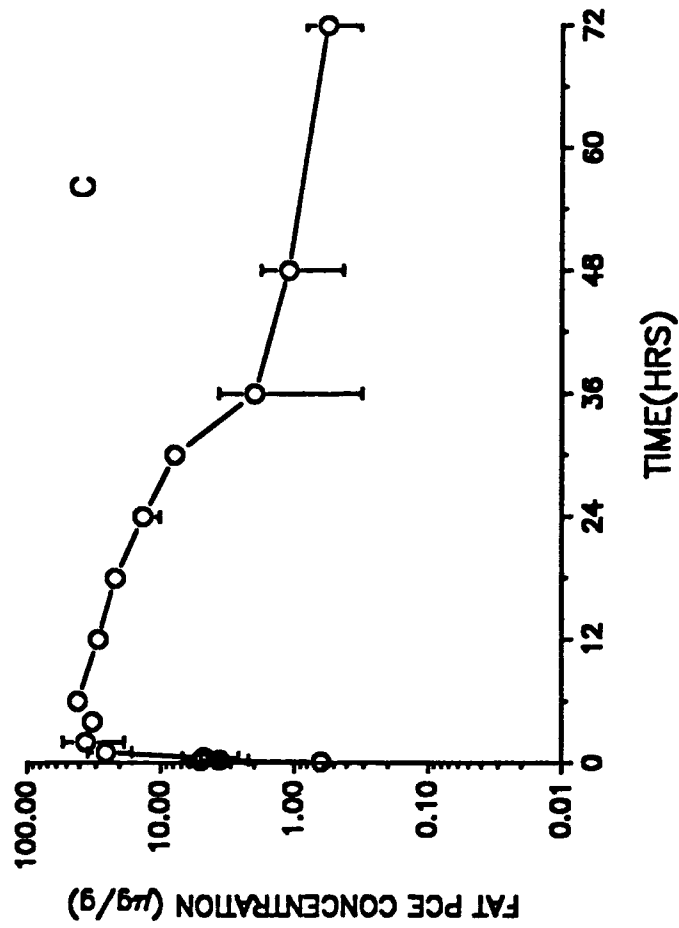
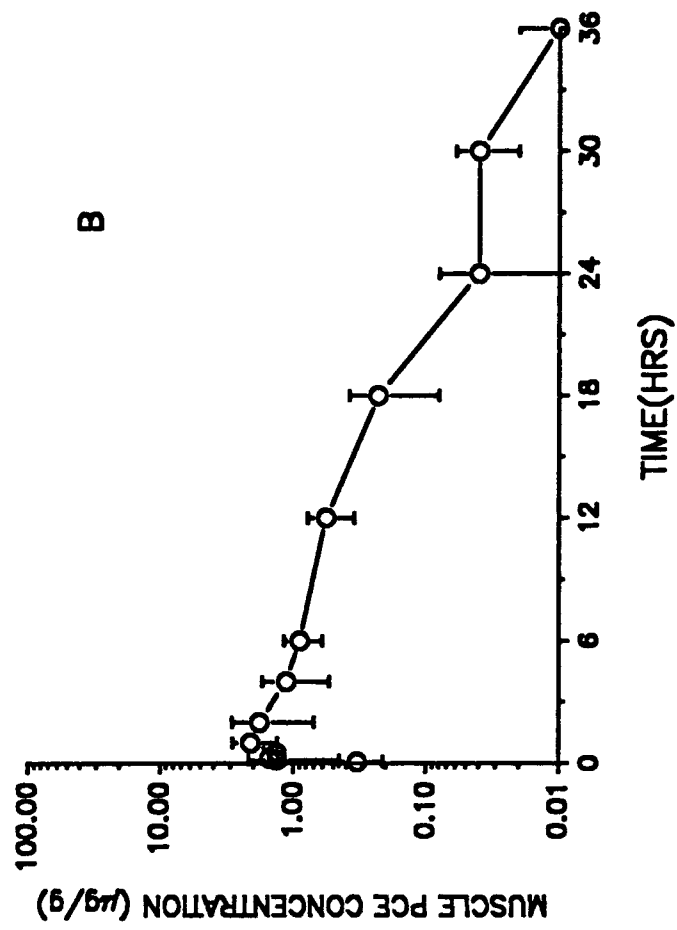
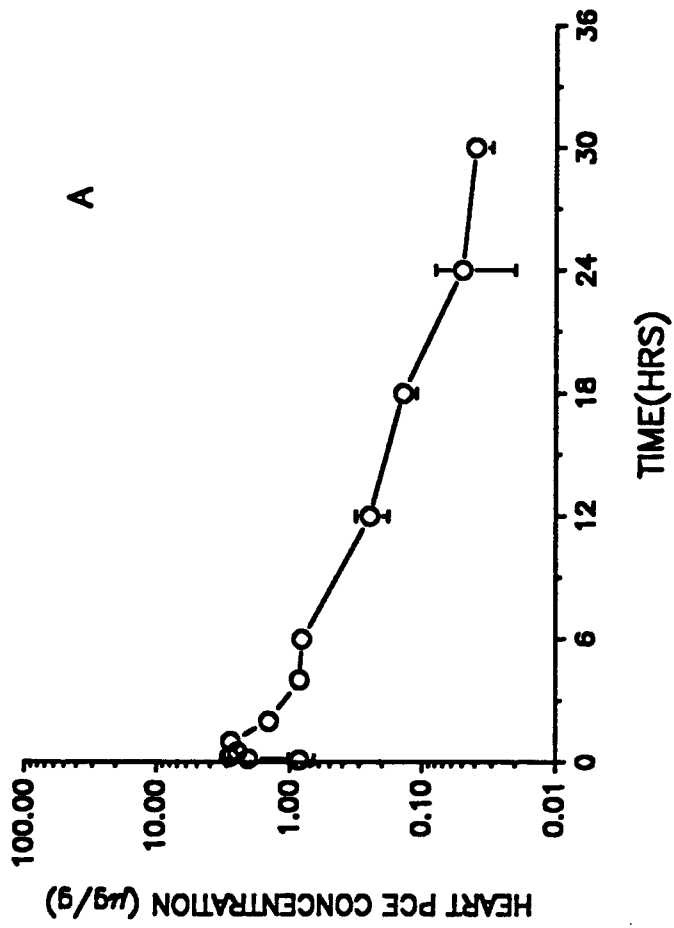
FIGURE LEGENDS

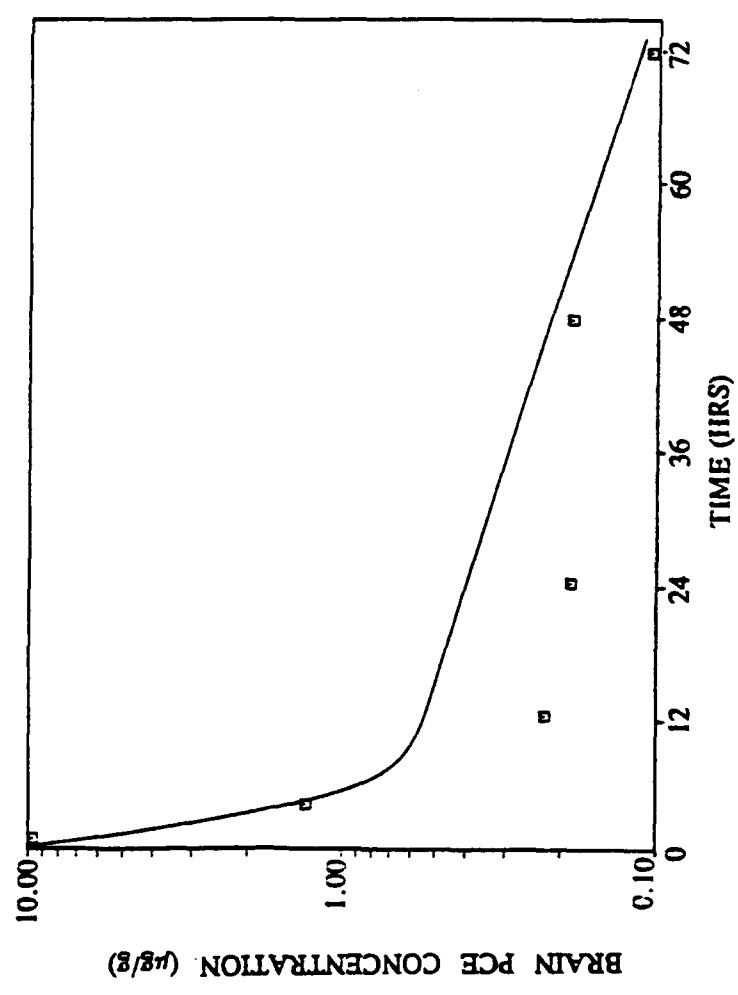
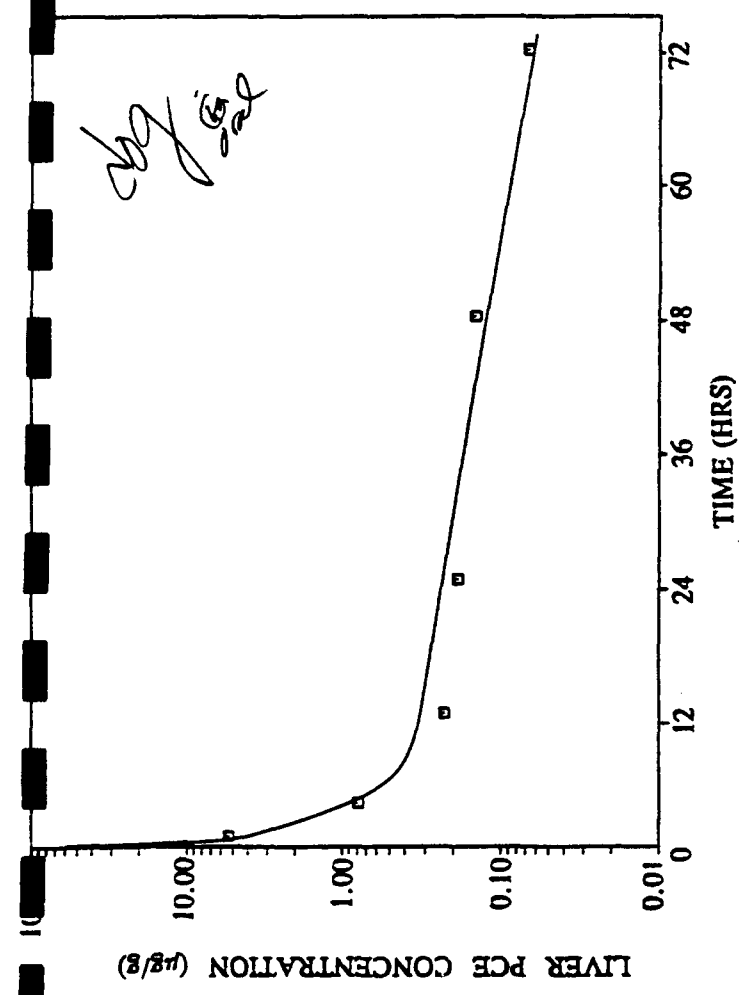
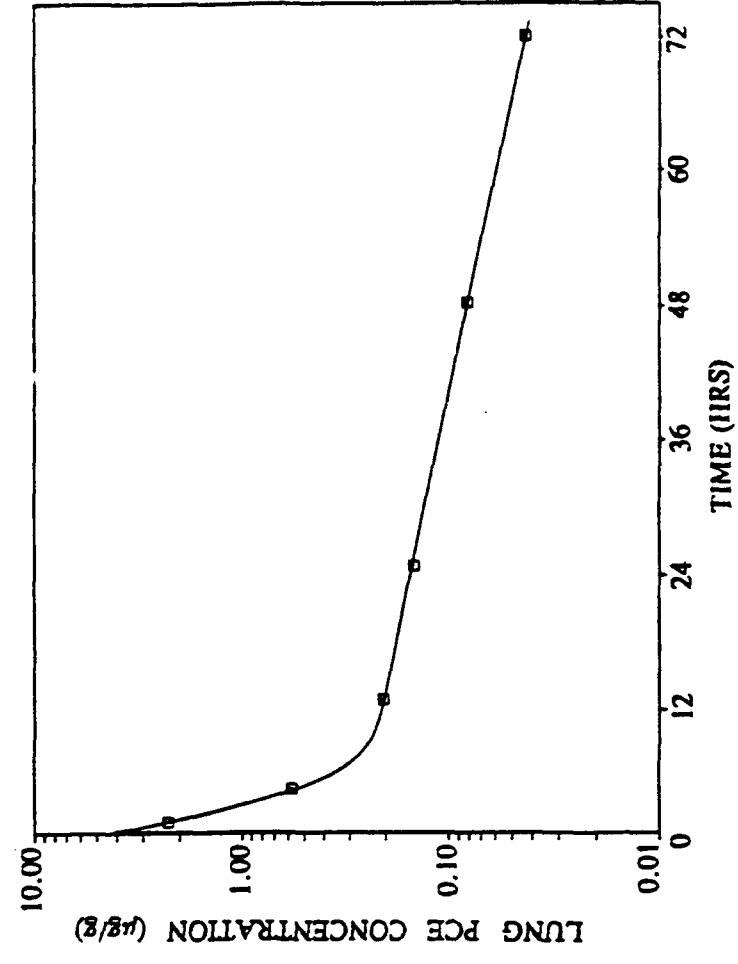
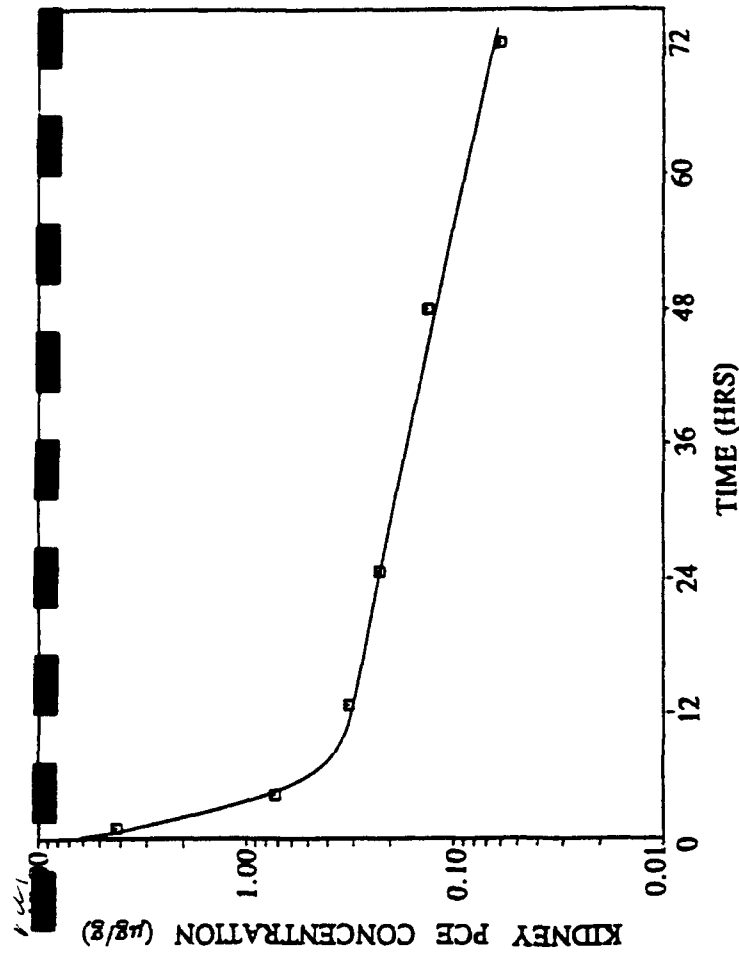
1. Diagram of the physiological pharmacokinetic model used to simulate the metabolism and exhalation of PCE following a) intraarterial administration to rats and following (b) oral administration to dogs. The parameters used to describe the model for ia administration in rats are taken from Dallas et al. (1993), and parameters for oral administration in dogs are included in Table 3.
2. PCE concentrations measured in the liver, kidney, brain, and lung of rats following administration of a single bolus of 10 mg/kg of PCE by gavage. Each point represents the mean \pm S.D. value for 4 rats.
3. PCE concentrations measured in the heart, muscle, fat and blood of rats following administration of a single bolus of 10 mg/kg of PCE by gavage. Each point represents the mean \pm S.D. value for 4 rats.
4. PCE concentrations measured in the liver, kidney, brain, and lung of dogs following administration of a single bolus of 10 mg/kg of PCE by gavage. Each point represents the mean \pm S.D. value for 4 dogs.
5. PCE concentrations measured in the heart, muscle, fat and blood of dogs following administration of a single bolus of 10 mg/kg of PCE by gavage. Each point represents the mean \pm S.D. value for 4 dogs.
6. Model predictions of exhalation and metabolism of PCE over time following ia administration of (a) rats po administration of (b) dogs with a single 10 mg/kg bolus dose of PCE.

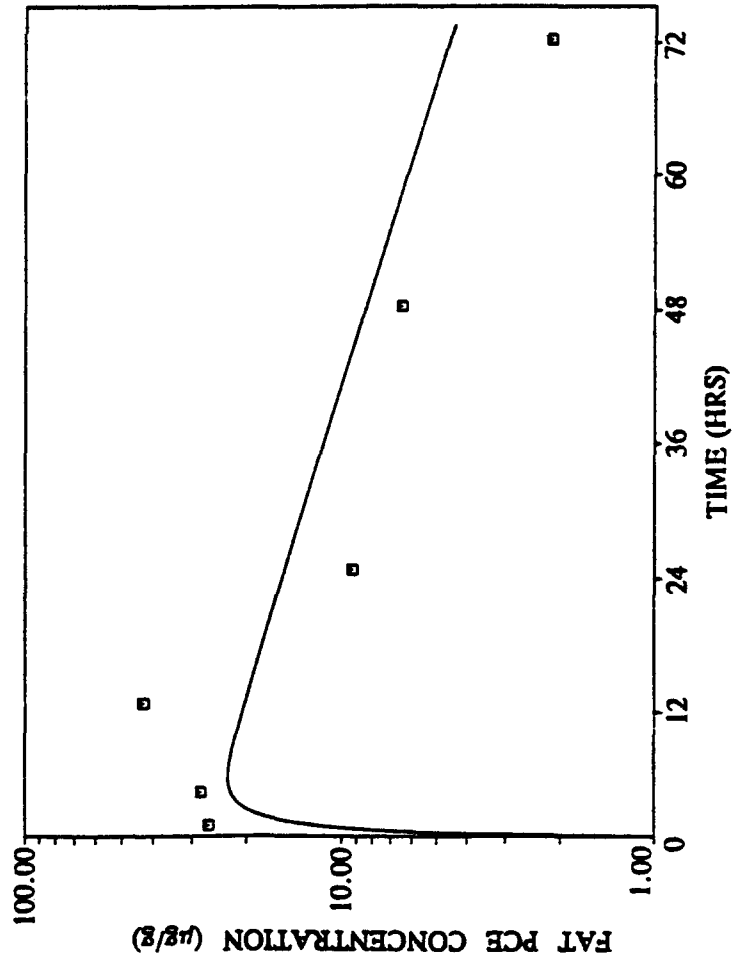
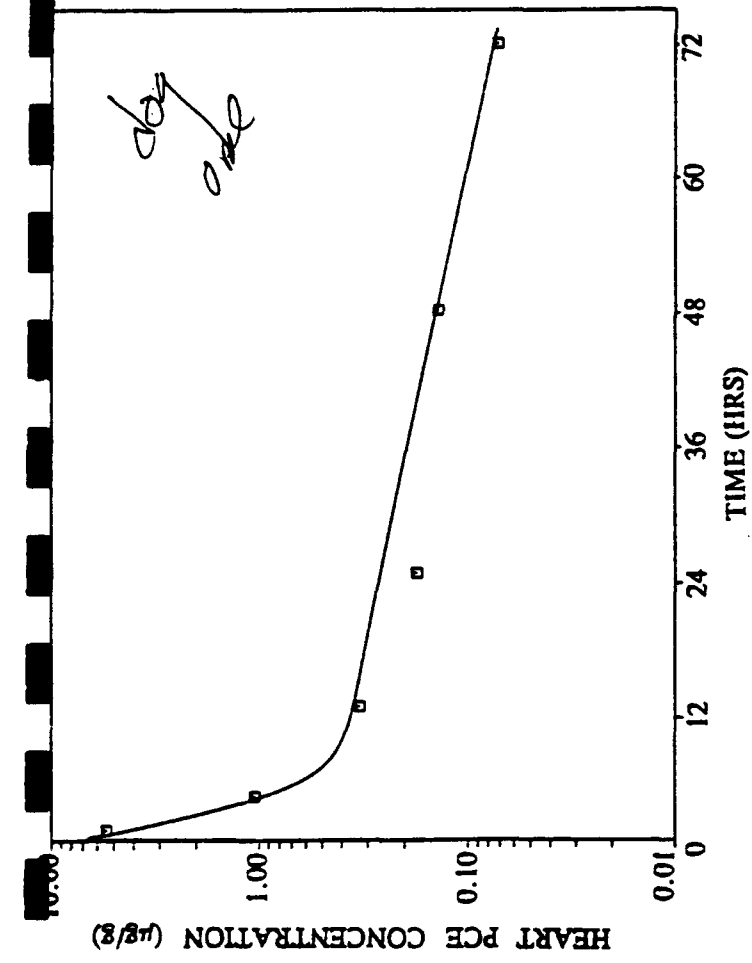
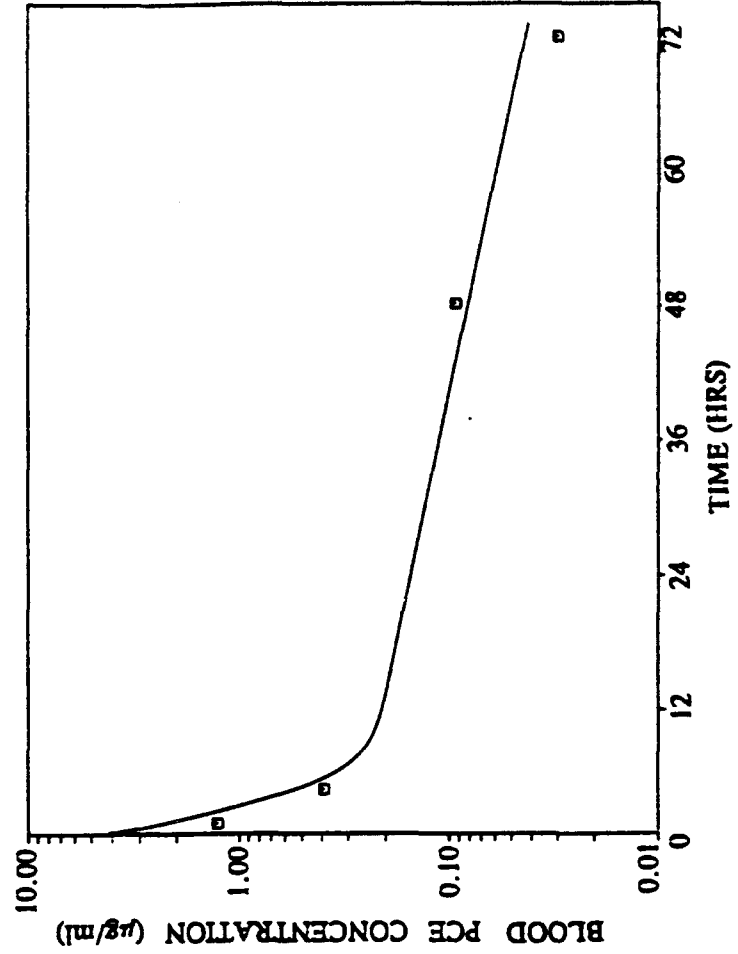
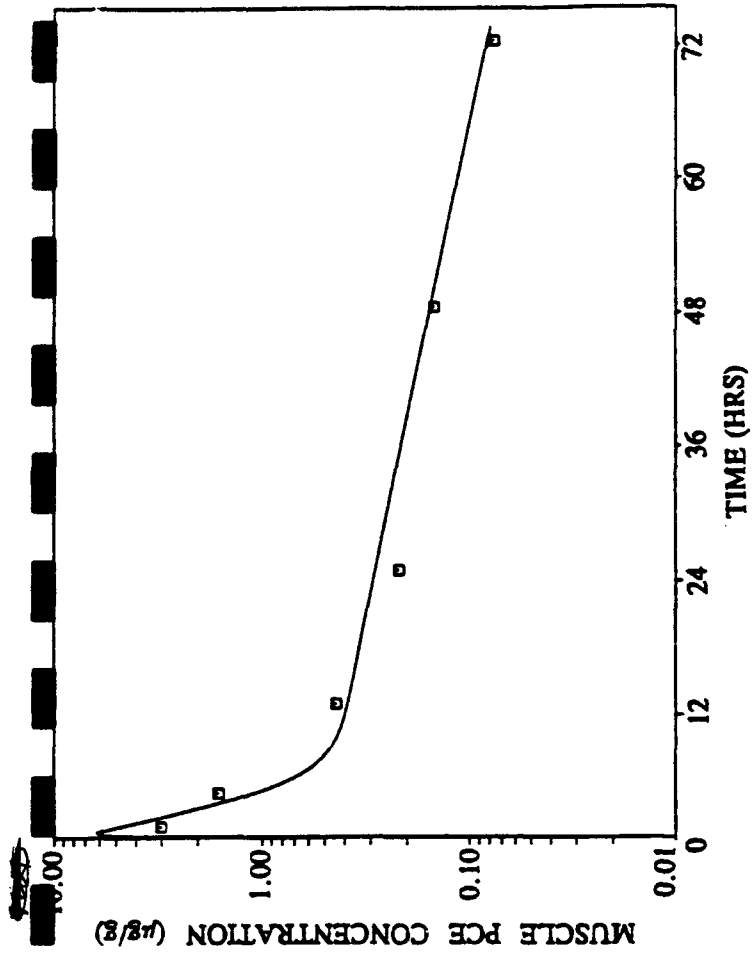
**PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL OF PCE
ORAL ADMINISTRATION**

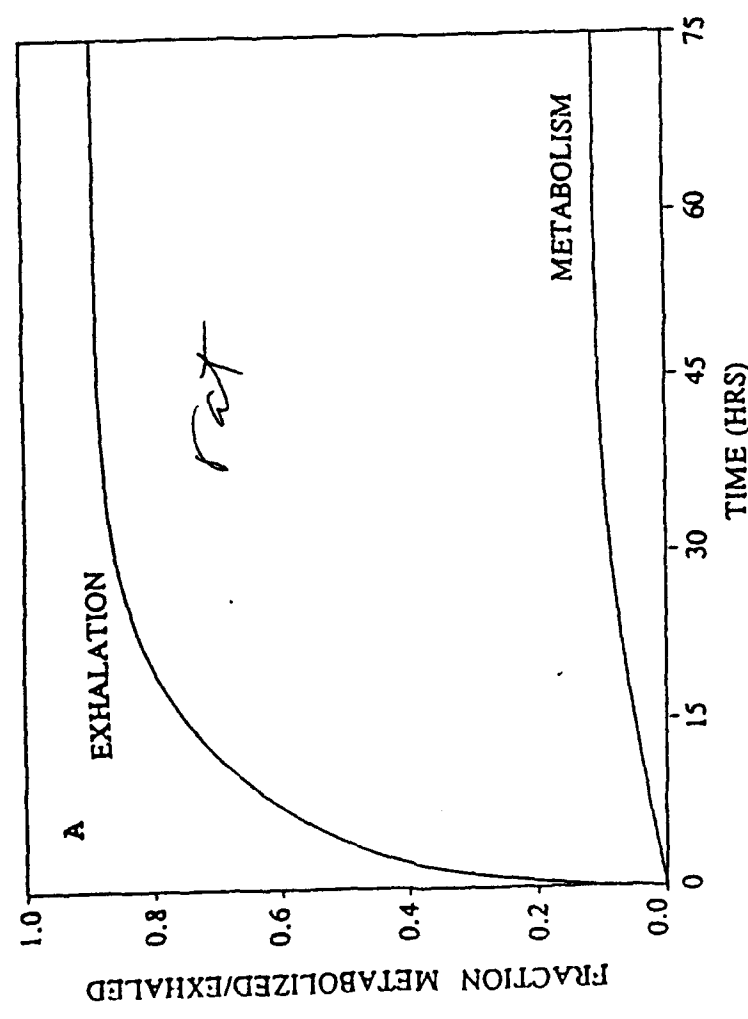
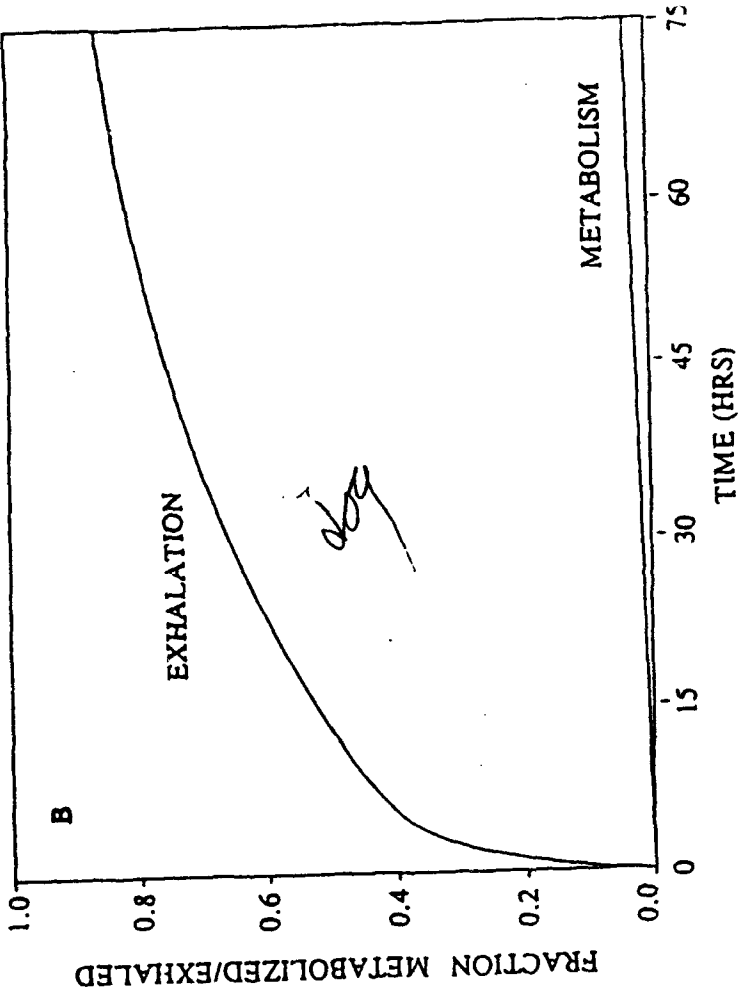












APPENDIX

SECTION E

Manuscript in preparation for submission to the
Journal of Toxicology and Environmental Health

Comparisons Between Species, Doses, and Routes of Administration
of Perchloroethylene Pharmacokinetics Using a Physiologically
Based Pharmacokinetic Model

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Comparisons of the pharmacokinetics of environmental chemicals between different species, dose levels, and routes of administration have been considerably enhanced by the use of physiologically-based pharmacokinetic (PBPK) models (Clewett and Andersen, 1985). Similarities in the anatomy and physiology of mammalian species make interspecies extrapolations (i.e., animal scale-up) possible (Dedrick, 1973; Boxenbaum, 1984). Pharmacokinetic models developed in a series of species may be scaled based on allometric relations to allow prediction of chemical concentrations in other species without the collection of additional experimental data. Model parameters such as tissue volumes and blood flows can be scaled based on allometric relationships which are functions of animal weight. Changing the input parameters for the concentration of chemical entering the exposed species allows for extrapolations between dose. Altering the point of entry of the chemical into the PBPK model enables pharmacokinetic comparisons between routes of administration using an otherwise similar modeling approach.

The accuracy of PBPK model predictions of perchloroethylene (PCE) pharmacokinetics have been evaluated by comparison to observed data under various experimental conditions. Ward et al. (1988) and Bois et al. (1990) predicted the rate of metabolite formation as related to a range of oral PCE dosage and compared the results to data in mice (Buben and O'Flaherty, 1985). Measurements of PCE metabolite formation in the urine of humans following a range of inhalation exposure concentrations (Ikeda et al., 1972) and PCE exhaled breath levels following different lengths of exposure were used to test the ability of a PBPK model to predict PCE pharmacokinetics over a range of inhalation dose measures (Ward et al., 1988). Comparisons in the effective received dose of PCE to mouse liver and lung between inhalation and drinking water ingestion have been

made using a PBPK model (Travis, 1987). PCE metabolite formation in mice, rats, and humans has been predicted using PBPK models (Bagen and McKone, 1988; Bois et al., 1990; Hattis et al., 1990). Exhaled breath levels of PCE and certain metabolic parameters have been predicted and compared to observed data for mice, rats, and humans (Chen and Blancato, 1987; Ward et al., 1988). In each of these studies, accuracy of model simulations was determined by comparisons to available data published in the literature.

A primary objective of the current study was to evaluate the accuracy of a PBPK model in predicting blood levels of PCE between species, exposure concentration and route of administration in comparison to observed data from matched experiments conducted for that purpose. Where possible, equivalent doses were administered in two species of wide variation in size, the rat and the dog, and a variety of doses used in each species. These exposures were repeated using two routes of administration, and venous blood levels over time were used to document the time course of PCE uptake, disposition, and elimination under each of the experimental regimens. The utility of the PBPK model predictions were then under a variety of experimental conditions.

METHODS

Male beagle dogs (5-10 kg), obtained from Marshall Farms (North Rose, NY), and male Sprague-Dawley rats (325-375 g), obtained from Charles River Laboratories (Raleigh, NC), were employed in these studies. The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. Rats were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow® were provided *ad libitum*. The rats were used after at least a 7-day acclimation

period, at which time they were approximately 12 weeks old. Dogs were housed in dog runs and also given a 14-day acclimation period, and were fed Purina Dog Chow®. Dogs were used between 6-9 months of age and in a weight range of 6-15 kg. solvent exposures were initiated between 1000 and 1200 hr each day.

1,1,2,2-Perchloroethylene (PCE) (tetrachloroethylene) of 99%+ purity was obtained from Aldrich Chemical Co. (Milwaukee, WI). The purity of the chemical during the conduct of the study was verified by gas chromatography.

For the animals intended to receive the ia dose, an indwelling carotid arterial cannula was surgically implanted the day before the exposure. For obtaining blood samples following compound administration, an indwelling jugular vein cannula was implanted into all the test animals. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml):acepromazine maleate (10 mg/ml):xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v/v/v). The cannulated animals were allowed relative freedom of movement during a 24-hr recovery period with the cannulas protected from manipulation by surgical tape at the back of the head. All animals employed in the kinetic studies were unanesthetized. PCE was administered as a single bolus, either ia or po at a dose of 10 mg/kg, using polyethylene glycol 400 volume/kg bw as a vehicle. Food was withheld during the 18 hour recovery period before dosing.

The rats and dogs were administered PCE at doses of 1, 3 or 10 mg/kg. PCE was administered as an emulsion of polyethylene glycol (PEG) in a single bolus dose. The ia administration was conducted using the carotid arterial cannula. Oral doses were administered using a gavage needle for rats and a teflon tube for dogs.

Serial 20 μ l blood samples were collected from an indwelling cannula in the jugular vein at intervals up to 96 hours following dosing. While rats exhibited only slight neurobehavioral effects following PER ia administration, the dogs receiving the 10 mg/kg ia dose demonstrated a very high degree of central nervous system (CNS) depression. Data for ia administration in dogs are therefore presented only for the 1 and 3 mg/kg dose. PCE concentrations in the blood of rats and dogs following 1 mg/kg oral administration rapidly declined below the limit of detection, so data for oral dosing in both species are shown only for the 3 and 10 mg/kg doses.

PER concentrations in the blood samples were analyzed by headspace gas chromatography (GC). The blood samples were transferred to 8-ml headspace vials. These vials were capped immediately with rubber septa and a spring washer and crimped to insure an airtight seal. Each sample vial was then placed into the autosampler unit of a gas chromatograph (GC). The limit of detection for PCE was approximately 1 ng, or 6.7 parts of PCE per billion parts of air in the sample vial.

A model Sigma 300 GC equipped with a HS-6 headspace sampler (Perkin Elmer Co., Norwalk, CT) was used for the analysis of the halocarbon. The GC was equipped with an electron capture detector. Analyses were carried out on stainless-steel columns (182 cm x 0.317 cm) packed with 10% FFAP (Alltech Associates, Deerfield, IL). The GC operating conditions were: Headspace sampler temperature, 90°C; injection port temperature, 200°C; column temperature, 110°C; detector temperature, 400°C; flow rate for argon/methane carrier gas, 60 ml/min. Each sample vial was heated thermostatically for 10 min in the GC autosampler unit, pressurized for 30 sec with the carrier gas, and injected automatically by a solenoid-controlled mechanism into the GC column.

The aforementioned conditions resulted in vaporization of the halocarbons in the sample vials, since PCE was heated to a temperature slightly below its boiling point, and the vial subsequently pressurized and vented into the GC.

The blood concentration-time data were evaluated by Lagran (M. Rocci and W.J. Jusko) computer programs for the assessment of the appropriate pharmacokinetic model and calculation of relevant pharmacokinetic parameters.

The disposition of PCE in the rat and the dog was predicted using a physiologically-based pharmacokinetic (PBPK) model for po (Fig. 1a) and ia (Fig. 1b) administration. It was similar to PBPK models previously developed by Angelo and Pritchard (1984) and Ramsey and Andersen (1984) for other VOCs, in that it provided for inhalation and exhalation through a lung compartment, metabolism in a liver compartment, and blood-flow-limited organ representations. The model for po administration differed from that for ia administration in the inclusion of a gastrointestinal (GI) compartment with a separate blood flow and a rate of oral absorption (k_a) dictating uptake of the PCE. Otherwise, the same model was used for both routes of administration. The current model differed primarily in that it included a greater number of tissue compartments. Values determined in this laboratory were employed for tissue volumes and blood flows (Delp et al., 1991) and alveolar ventilation (Dallas et al., 1991) for the male Sprague-Dawley rat. *In vivo* tissue:blood partition coefficients were calculated from ia data (rat) or oral data (dog) obtained in the present study by the area method of Gallo et al., (1987). The metabolic parameters K_m and V_{max} , and blood:air coefficients were estimated from the observed data by nonlinear regression analysis. Parameters f and K_a were estimated from oral data by fitting of the blood and liver concentration versus time data for both species. Tissue volumes and blood flows for the dog were obtained from the literature. Sources of tissue volumes

were as follows: liver, heart, lung, muscle, and blood (Andersen 1970), kidney (Spector, 1956), and fat (Sheng and Huggins, 1971). Sources of blood flows were as follows: liver (Liang et al., 1982), kidney, muscle, and brain (Humphrey and Zins, 1983), cardiac output (Andersen, 1970), fat (Nagasaka et al., 1976), and heart (Liard et al., 1982). Values for alveolar ventilation in the dog were taken from the publication by Andersen (1970).

The area under the concentration-time curve (AUC) for blood and tissues was determined from the time of administration to infinity. Total body clearance was determined as the dose divided by the blood AUC in each species. Bioavailability was calculated by AUC_{po}/AUC_{ia} . The maximum concentration reached in blood and tissues (C_{max}) and the time after dosing that it occurred (T_{max}) were determined by observation of the available data points.

Differential mass balance equations, incorporating the parameters listed in Table 1, that described the transport of PCE in the rat and the dog were numerically integrated with the Advanced Continuous Simulation Language (ACSL)

RESULTS

Comparisons between the pharmacokinetic parameters following different doses and routes of administration are shown in Table 2 for rats and Table 3 for dogs. Following oral administration in rats, the maximum tissue concentration (C_{max}) was achieved between 20 and 40 minutes at all three doses. The AUC achieved was approximately proportionate to the three po doses employed in the rat and to the two ia doses employed. The $t_{1/2}$ was significantly longer in the po-dosed rats than in those receiving ia administration at a dose of 3 mg/kg ($p < 0.05$) and at 10 mg/kg ($p < 0.01$).

The AUC was not proportionate to dose in dogs, with a 2-fold difference between 3 and 10 mg/kg doses for ia administration and a 4.3-fold difference between the 1 and 3 mg/kg po doses. The AUC was therefore approximately 50% lower and higher than expected for the ia and po dose comparisons, respectively. The C_{max} achieved following ia administration was 7 times higher for the 3 mg/kg dose than for 1 mg/kg, and 50% lower than would be expected for a proportional comparison between the 3 and 10 mg/kg po doses. The t_½ values for po and ia administrations at the same dose (3 mg/kg) were not significantly different.

For an equivalent dose and route of administration, the AUC in dogs was essentially twice that observed in rats. Comparisons of t_½ between species reveal significantly higher values ($p < 0.001$) for po and ia administrations at the 3 mg/kg dose, but there was not a significant difference between species at the 10 mg/kg po dose. The C_{max} achieved in dogs was approximately 3.1-3.5 times that in rats for an equivalent ia and po dose at 3 mg/kg, but was not significantly different at the 10 mg/kg dose. Bioavailability of PCE was essentially 100% at the 3 mg/kg dose in dogs, and slightly lower in rats.

Predictions of PCE blood concentrations over the time following ia administration are compared to experimentally determined (i.e., observed) values for three doses in the rat in Fig. 2. Over the 10-fold range of doses employed, concentrations of PCE in the venous blood were well predicted by the model. A slight overprediction during the initial phase of rapid decline in the blood was followed by model-generated concentrations that were in close agreement with the observed values during the terminal elimination phase at all three doses.

Observed and predicted concentrations in the venous blood of dogs are shown for ia administration with PCE in Fig. 3. A consistent overprediction of PCE blood concentration is shown relative to the observed data at the 3 mg/kg dose.

The same is true for the 1 mg/kg dose during the rapid elimination phase, though the potential for somewhat closer predictions in the terminal elimination phase are obscured by the inability to completely procure samples past 10 hour due to the limit of detection of the assay.

Model-generated and observed blood PCE concentrations were compared for three doses following oral administration in the rat (Fig. 4). At all three doses there was a pattern of a slight overprediction at the peak blood levels that was followed by observed values that were in close agreement with the predictions during the initial part of the rapid elimination phase. Later predictions during the time course were overpredicted, except for the terminal elimination phase at the highest dose employed (10 mg/kg) where there was quite good agreement with the observed values.

Following oral administration of PCE in the dog, model-predicted concentrations were higher than the observed values for both concentrations during the rapid elimination phase for which data were available (Fig. 5). During the terminal elimination phase, predicted PCE blood concentrations were increasingly overpredicted over the time course at the 10 mg/kg dose. Following the 3 mg/kg dose, however, model predictions were in fairly close agreement with the observed values.

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Table 1
Parameters Used in the Physiologically Based
Pharmacokinetic Model for PCE in the Rat and the Dog

Parameter	Rat	Dog
Body Weight (kg)		
<hr/>		
Tissue volumes (ml)	Percentage of Body Weight	
liver	3.39	4.22
kidney	0.77	0.6
fat	5.0	15.2
heart	0.33	1.13
lung	0.37	0.66
muscle	35.36	46.8
brain	0.6	0.75
blood	7.40	8.19
rest of body	46.78	22.45
Alveolar ventilation (ml/min)	1.54 (ml/min·g) BW(g) ^{0.75}	2.576 (ml/min·g) BW(g) ^{0.75}
Cardiac output	1.57 (ml/min·g) BW(g) ^{0.75}	1.05 (ml/min·g) BW(g) ^{0.75}
<hr/>		
Percentage of Cardiac Output		
Blood flows (ml/min)		
liver	15.73	5.12
kidney	13.13	10.1
fat	6.56	5.02
heart	4.73	3.37
lung	100	100
muscle	26.11	40.59
brain	2.21	3.12
blood	100% = 1.57 (ml/min·g) BW(g) ^{0.75}	100% = 2.05 (ml/min·g) BW(g) ^{0.75}
rest of body	31.53	32.68
<hr/>		
Partition Coefficients		
Blood:air	19.6	40.5
Fat:blood	152.5	63.2
Lung:blood	2.47	0.97
Liver:blood	5.25	1.64
Muscle:blood	2.98	1.9
Brain:blood	4.37	2.66
Heart:blood	2.68	1.74
Kidney:blood	4.45	1.49
Rest of body:blood	2.98	1.9
<hr/>		
Metabolism constants		
V _{max} (μg/min)	0.15	0.85
K _m (μg/ml)	0.019	0.023

TABLE 2

PHARMACOKINETIC PARAMETERS IN THE RAT¹

Dose (mg/kg)	Route ²	N	Area Under Curve ($\mu\text{g}\cdot\text{min}/\text{ml}$)	Biological Half-Life (Hrs)	Cmax ($\mu\text{g}/\text{ml}$)	Tmax (min)	Bioavailability (F)	Clearance ($\text{ml}\cdot\text{min}/\text{kg}$)
1	PO	6	27.88 \pm 1.67	3.24 \pm 0.21	0.17 \pm 0.02	20.83 \pm 2.00	0.96	36.69 \pm 2.76
1	IA	6	29.17 \pm 2.27	3.78 \pm 0.52	0.59 \pm 0.2	1.25 \pm 0.43		34.84 \pm 2.39
3	PO	5	100.48 \pm 10.15	7.85 \pm 0.96	0.38 \pm 0.11	37 \pm 23.88	0.94	30.11 \pm 2.95
3	IA	5	107.31 \pm 16.81	5.98 \pm 1.19	1.29 \pm 0.23	2.8 \pm 1.64		28.49 \pm 4.29
10	PO	6	321.37 \pm 27.56	15.49 \pm 1.46	1.58 \pm 0.23	22.5 \pm 4.79	0.82	32.52 \pm 3.31
10	IA	4	391.60 \pm 36.37	7.51 \pm 1.67	4.08 \pm 0.4	6 \pm 0.82		26.23 \pm 2.48

¹Each value represents the mean \pm s.e., except Tmax.²PO is oral administration, and IA is intraarterial administration

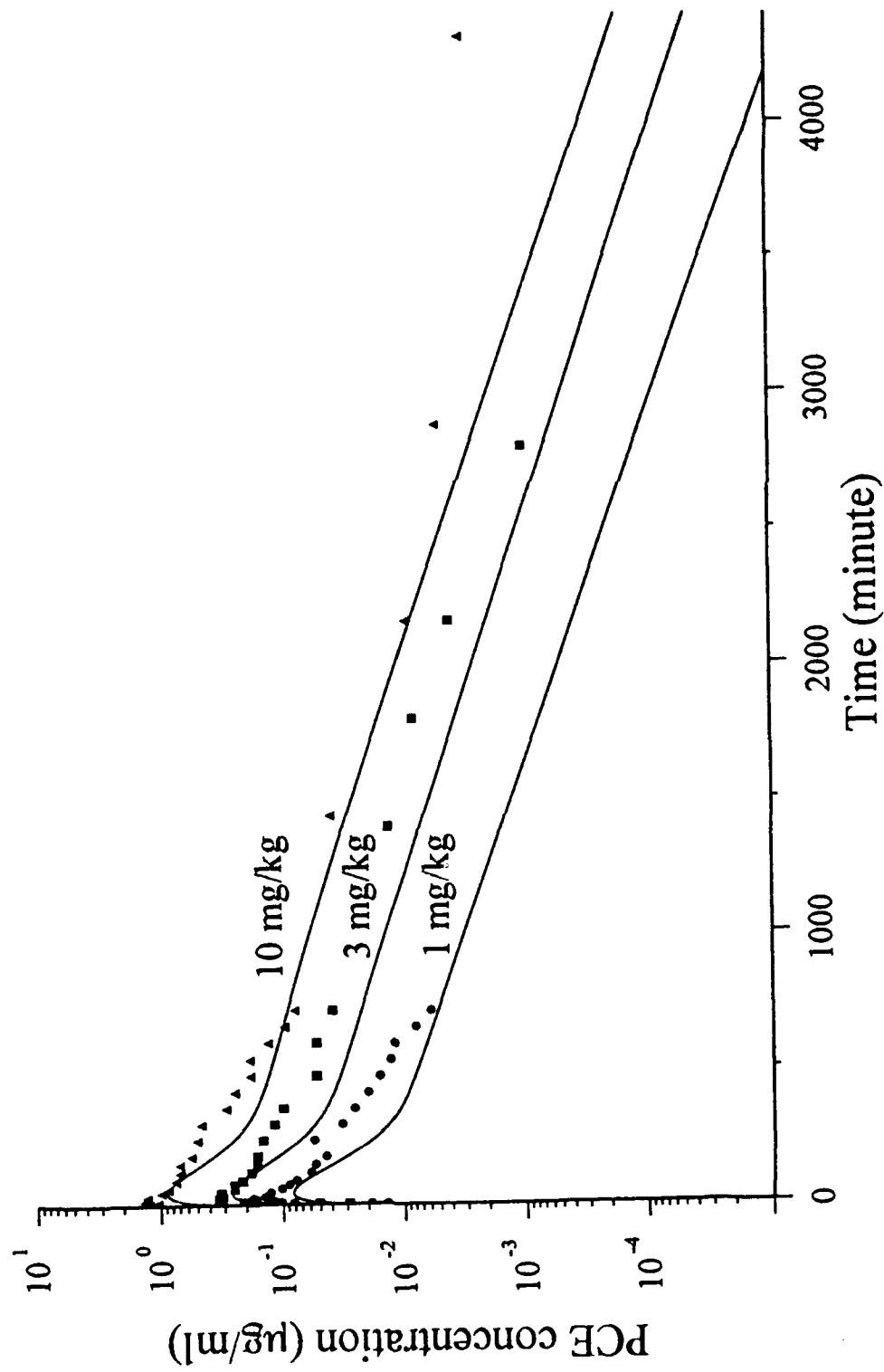
TABLE 3

PHARMACOKINETIC PARAMETERS IN THE DOG¹

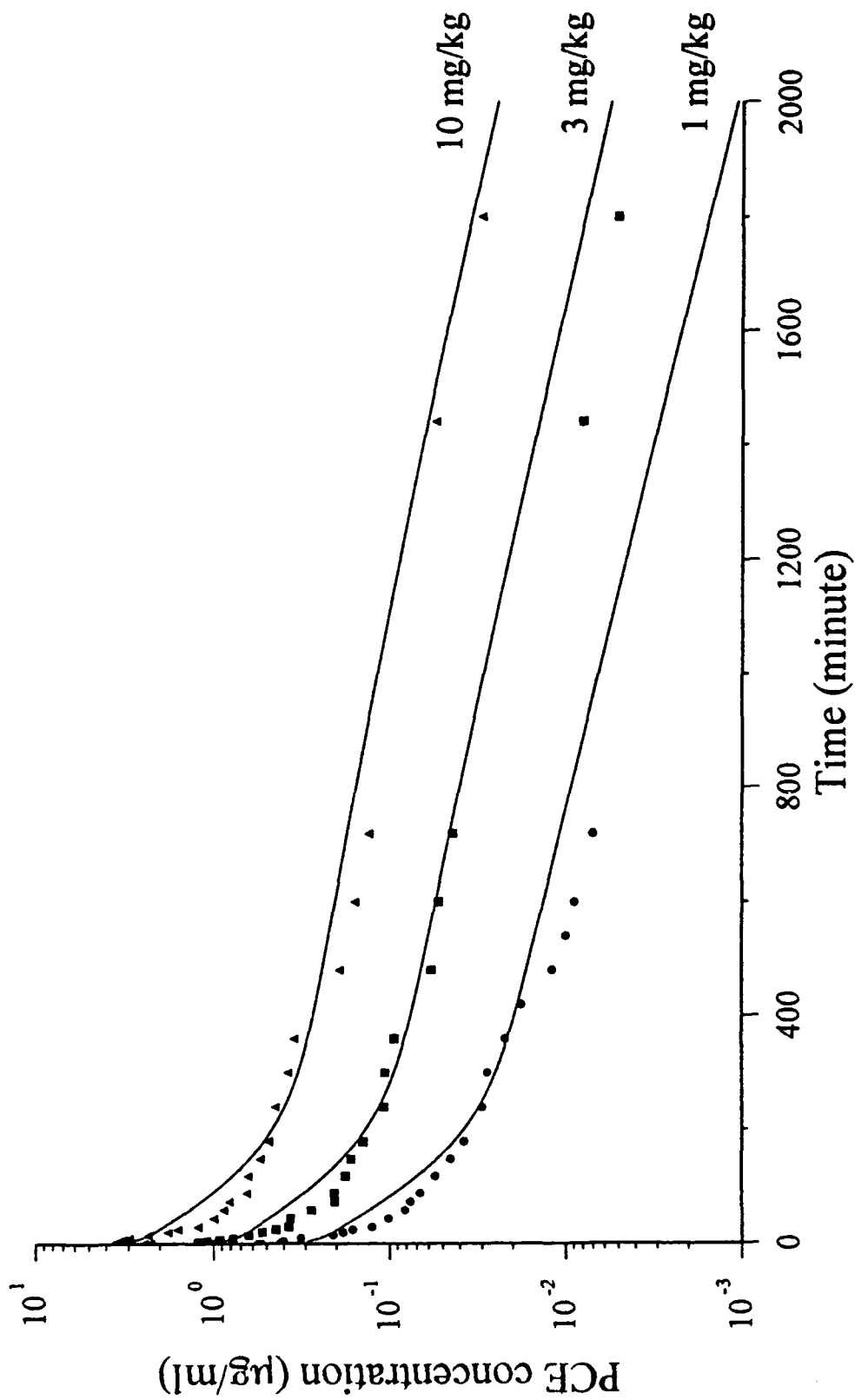
Dose (mg/kg)	Route ²	N	Area Under Curve ($\mu\text{g}\cdot\text{min}/\text{ml}$)	Biological Half-Life (Hrs)	Cmax ($\mu\text{g}/\text{ml}$)	Tmax (min)	Clearance ($\text{ml}\cdot\text{min}/\text{kg}$)
1	IA	4	50.29 ± 11.34	6.43 ± 0.44	0.64 ± 0.15	5.5 ± 1.71	23.02 ± 4.66
3	PO	4	214.25 ± 25.41	20.76 ± 2.16	1.21 ± 0.19	15 ± 0	14.65 ± 1.83
3	IA	3	213.98 ± 17.39	21.65 ± 1.61	4.52 ± 1.02	2.33 ± 0.72	14.29 ± 1.12
10	PO	8	451.06 ± 51.22	16.61 ± 1.39	1.93 ± 0.11	31.0 ± 9.89	25.01 ± 3.88

¹Each value represents the mean \pm s.e., except Tmax²PO is oral administration, and IA is intraarterial administration

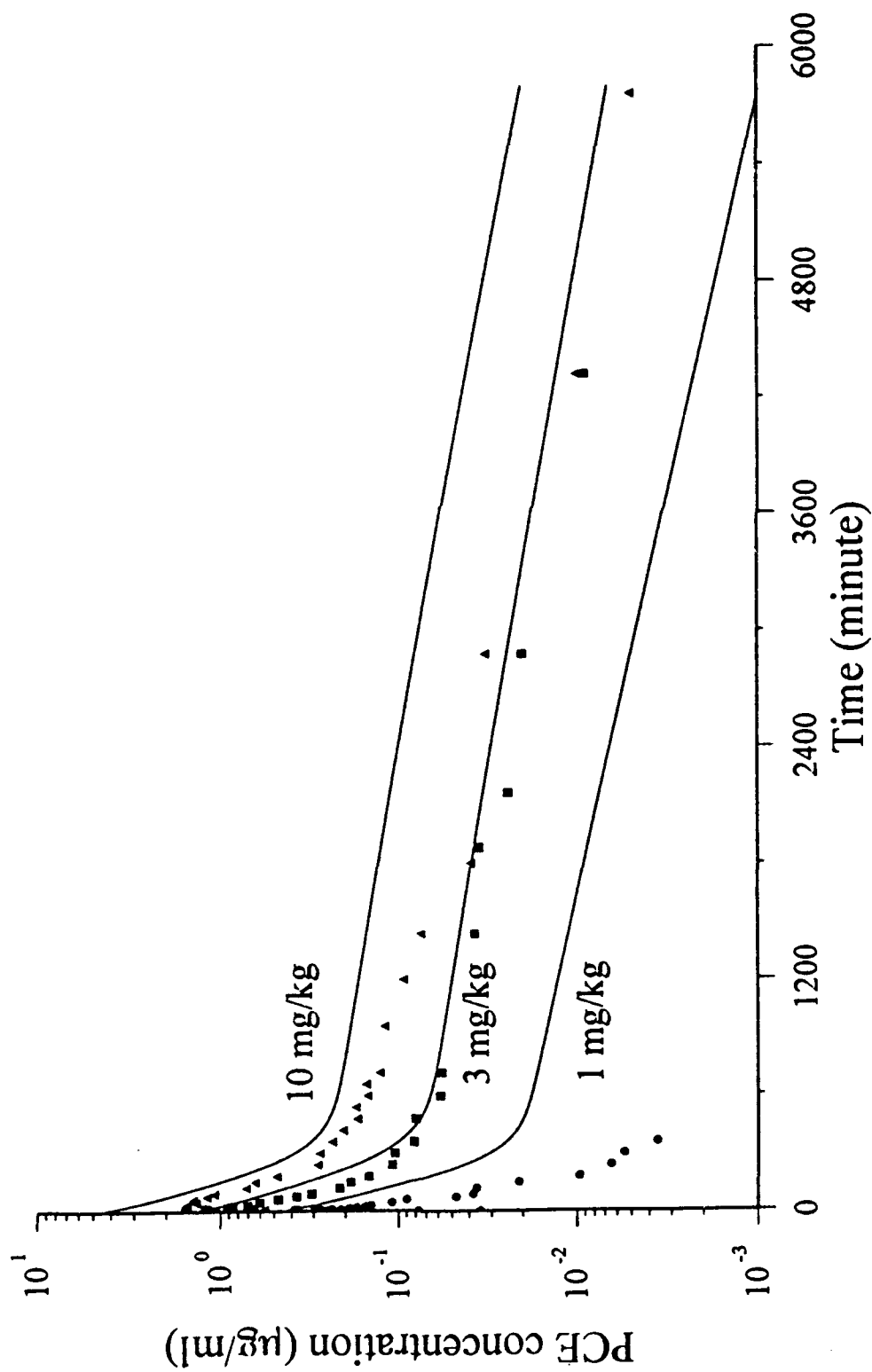
PCE concentration in venous blood after oral administration in rat



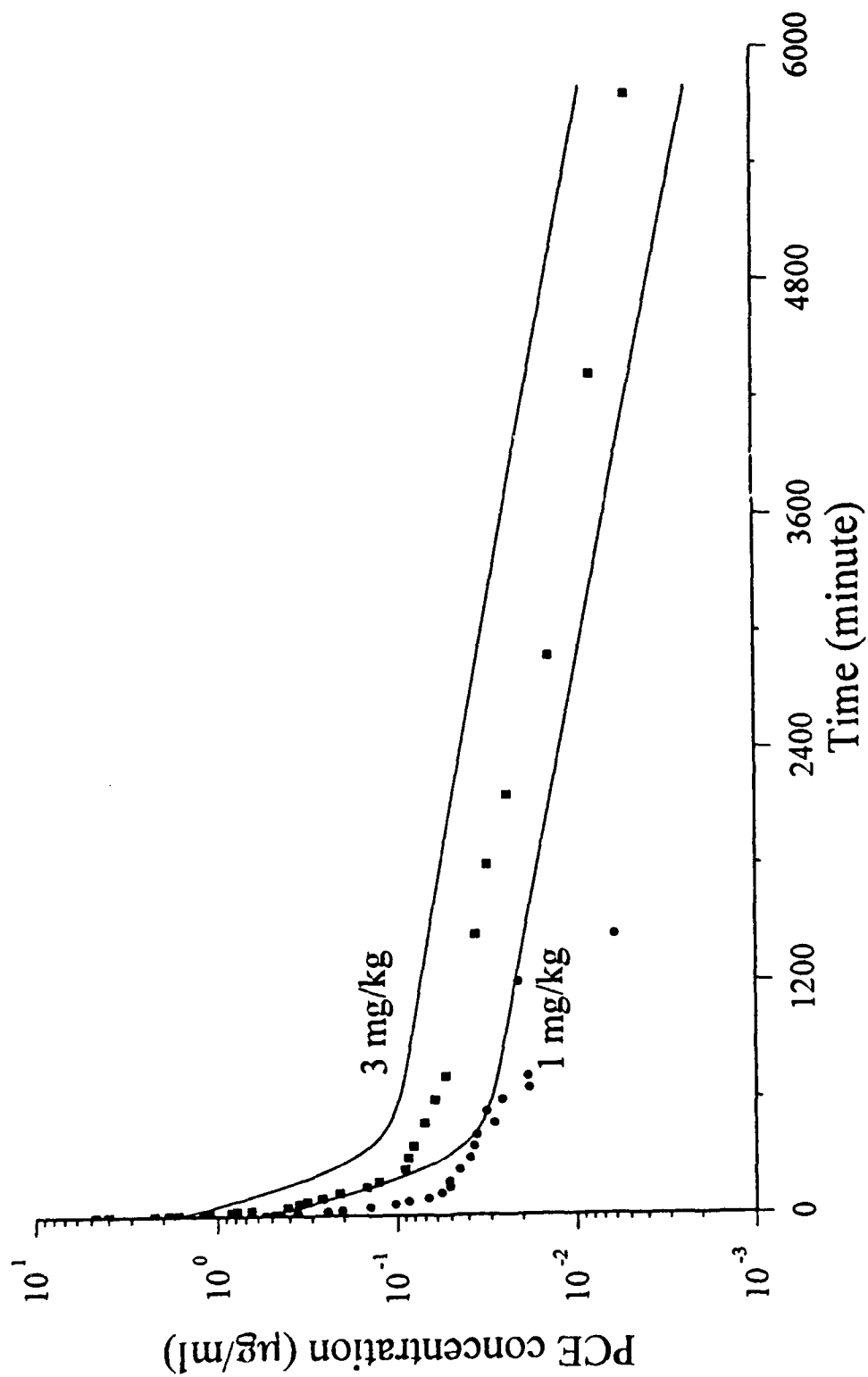
PCE concentration in venous blood after i.a. administration in rat



PCE concentration in venous blood after oral administration in dog



PCE concentration in venous blood after i.a. administration in dog



APPENDIX

SECTION F

TISSUE-CONCENTRATION TIME COURSE

DATA FOR TET IN RATS AND DOGS

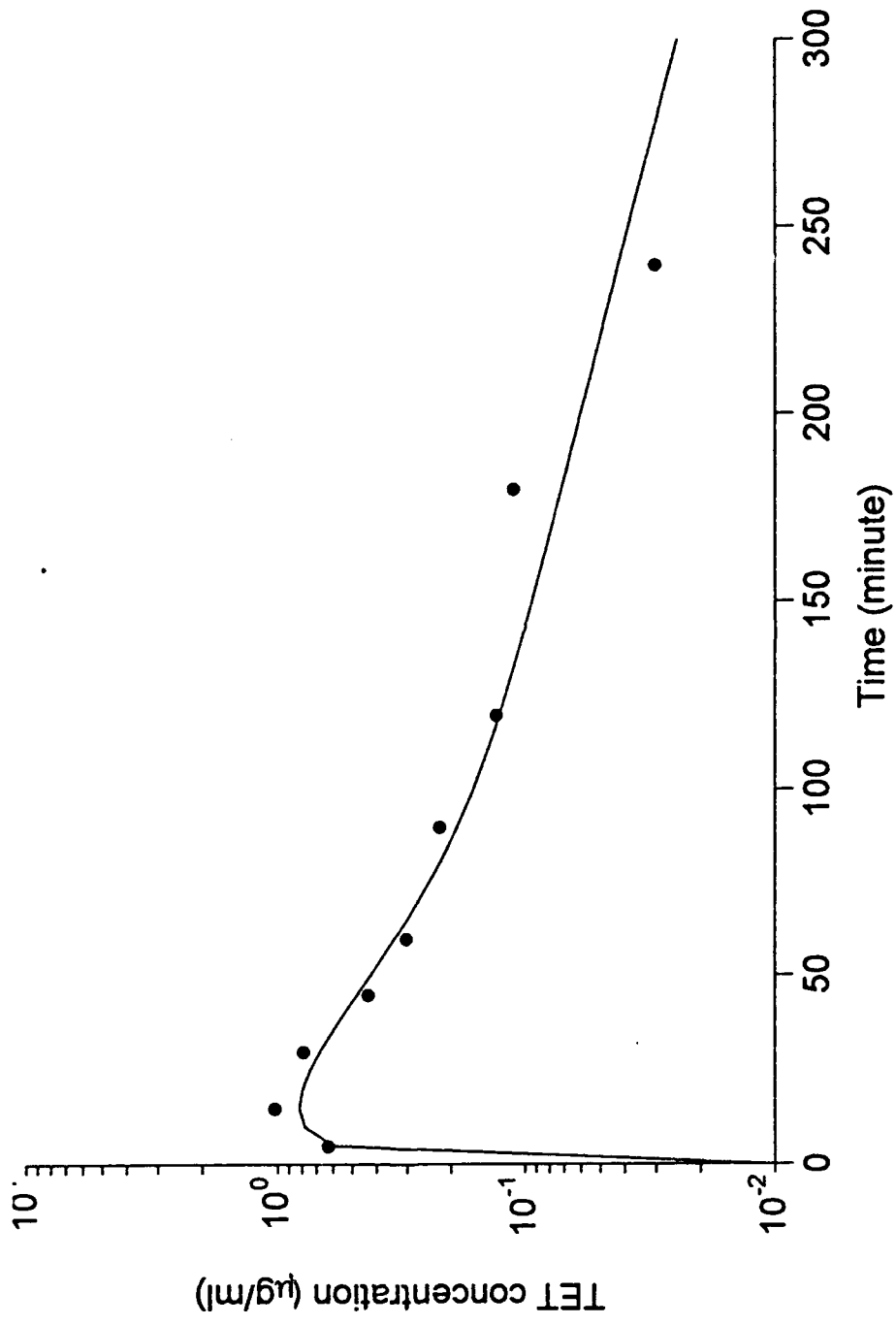
PHARMACOKINETIC PARAMETERS
TET INTRAARTERIAL ADMINISTRATION (10 MG/KG): RATS

	AUC ug • min/g	t½ min	Cmax ug/g
LIVER	794.3	97.0	20.0
KIDNEY	829.8	93.7	26.1
FAT	10277.2	164.0	65.0
HEART	650.5	105.7	21.9
LUNG	400.9	89.6	8.5
MUSCLE	381.1	106.1	4.7
BRAIN	510.0	83.9	12.9
BLOOD	248.3	70.5	5.5

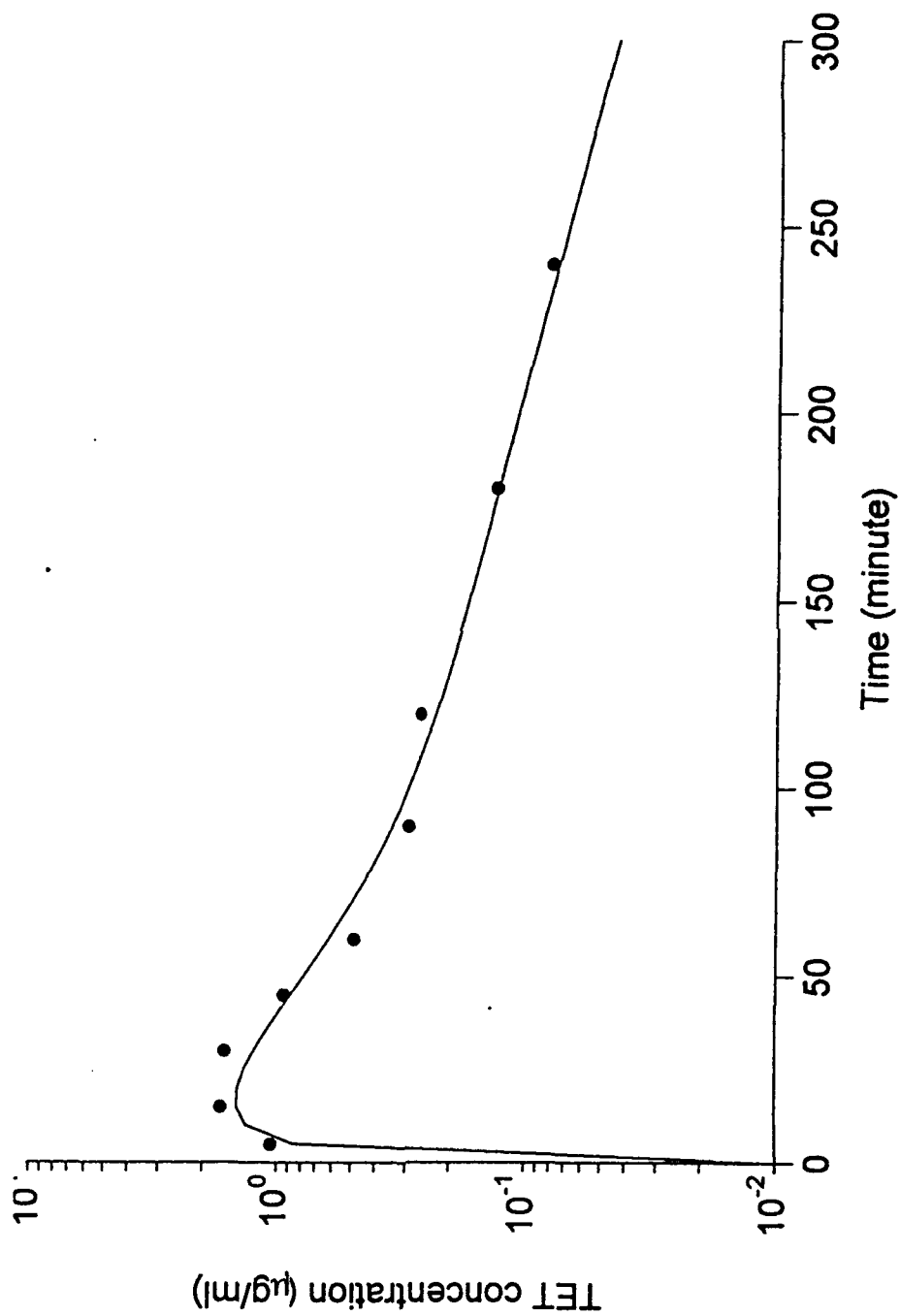
TET ORAL ADMINISTRATION (10 mg/kg) = RATS

	AUC ug • min/g	t½ min	Cmax ug/g
LIVER	290.0	61.8	4.8
KIDNEY	131.2	63.6	1.8
FAT	2108.3	216.0	6.1
HEART	111.6	65.8	1.8
LUNG	77.8	49.0	1.4
MUSCLE	75.2	91.1	0.8
BRAIN	116.3	71.5	1.7
BLOOD	65.5	58.6	1.0

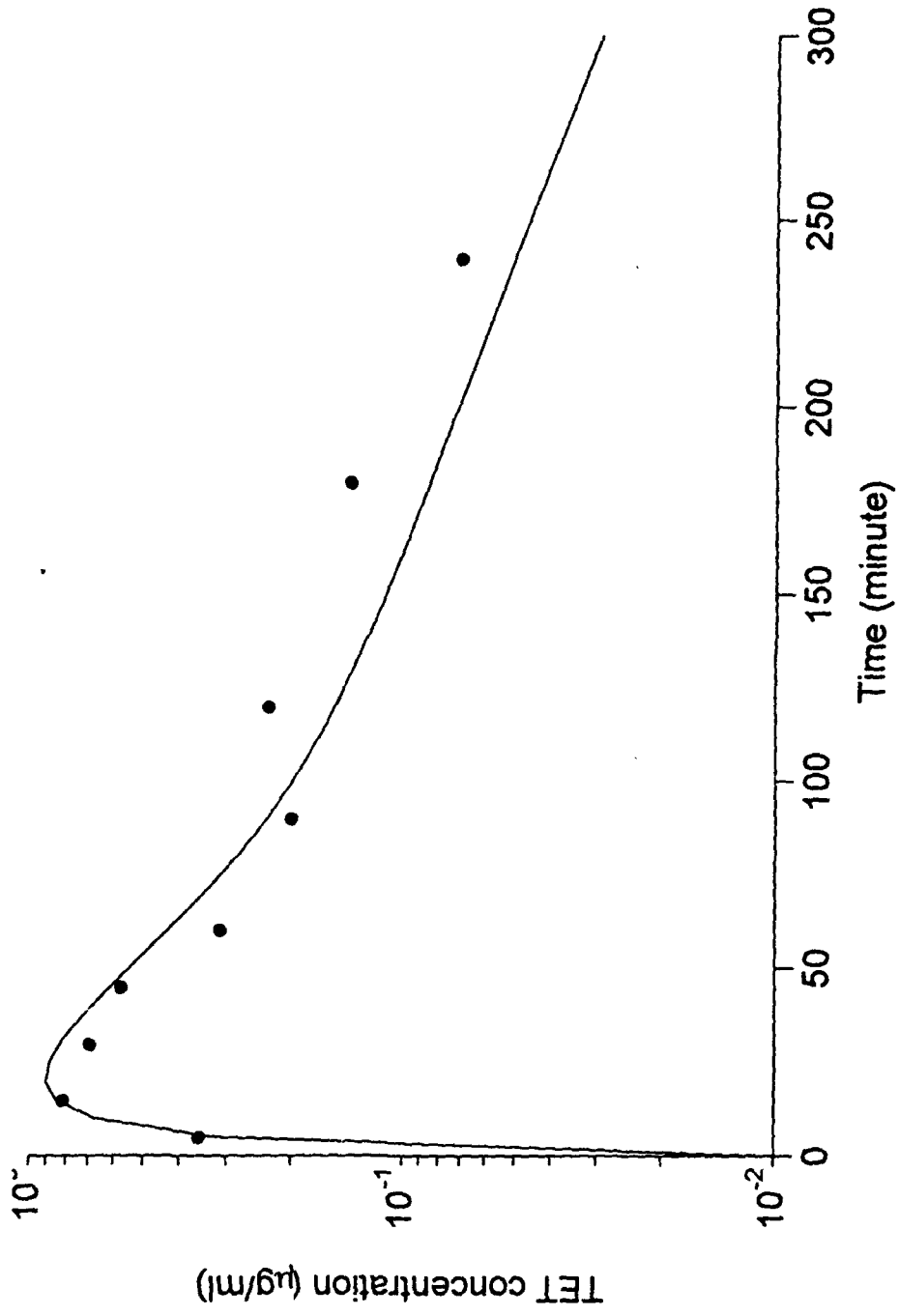
TET concentration in arterial blood after oral administration in rat



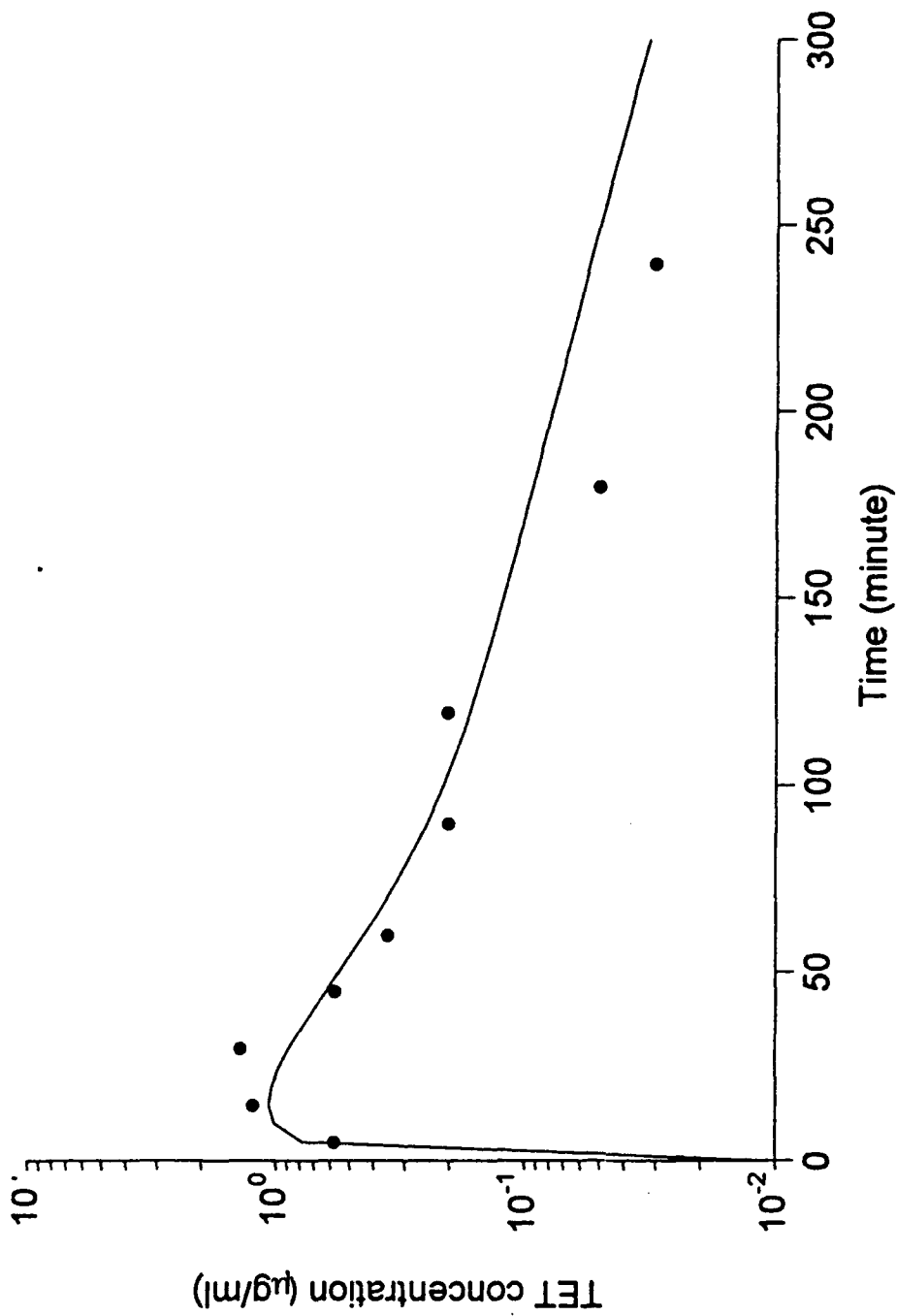
TET concentration in brain after oral administration in rat



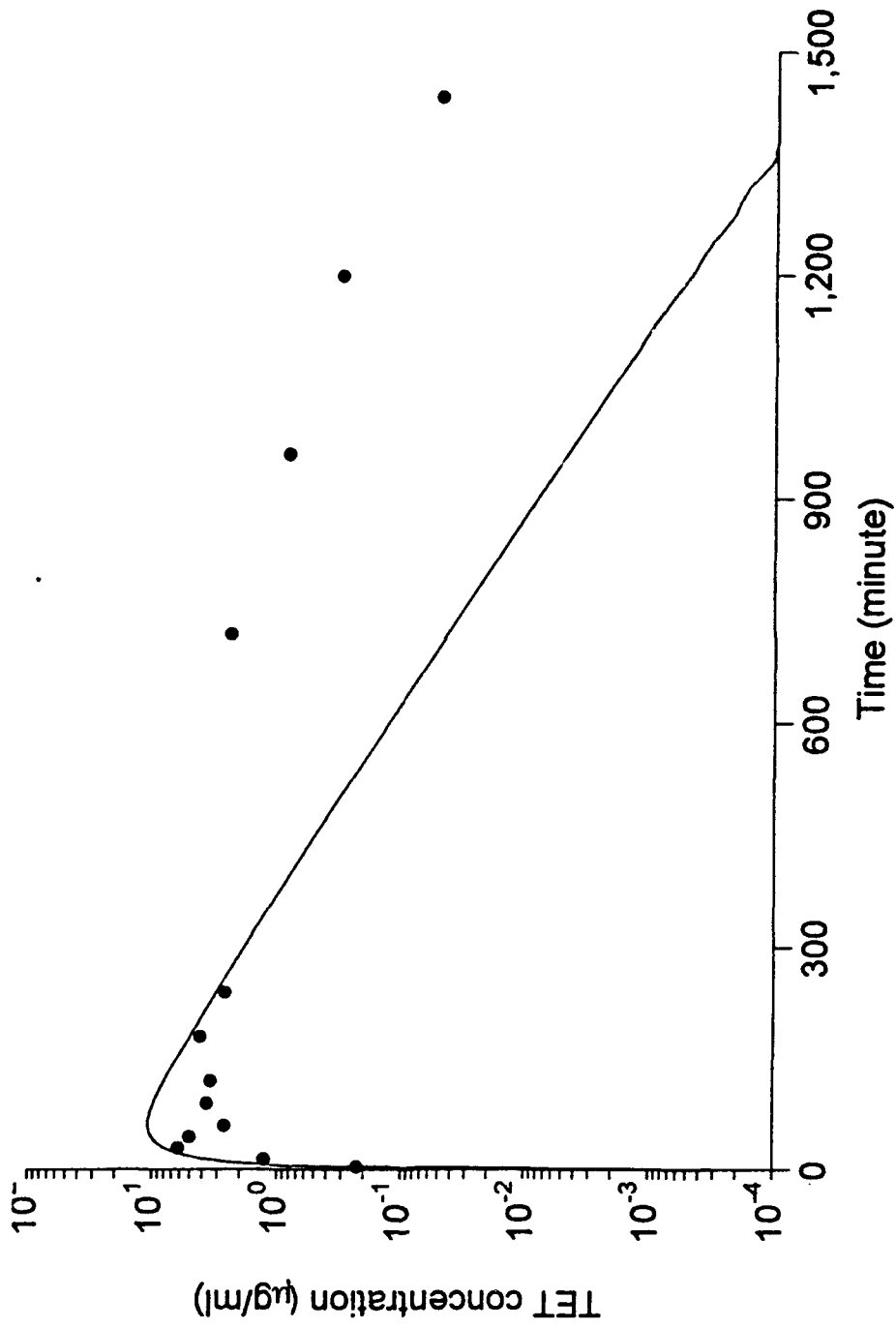
TET concentration in muscle after oral administration in rat



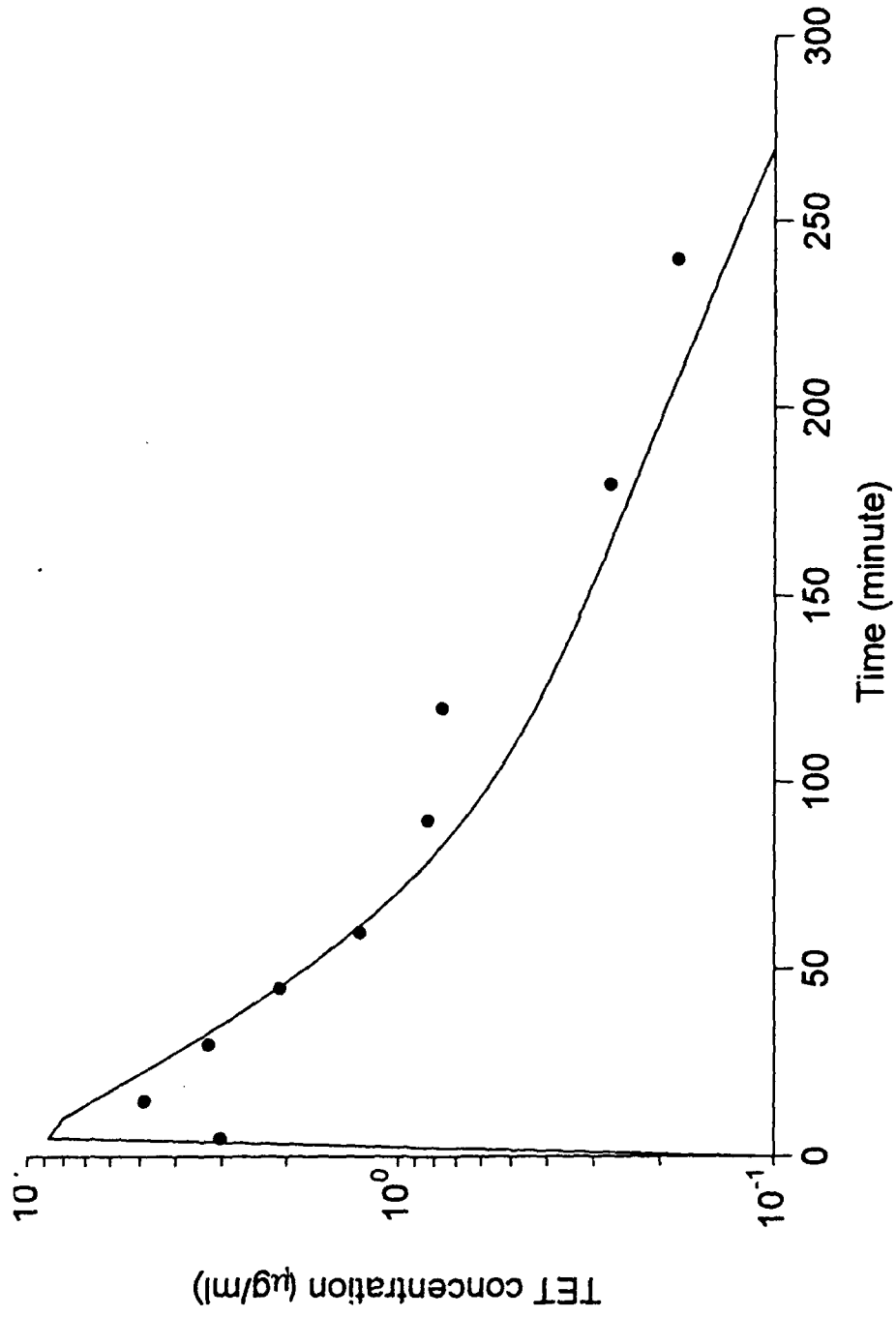
TET concentration in lung after oral administration in rat



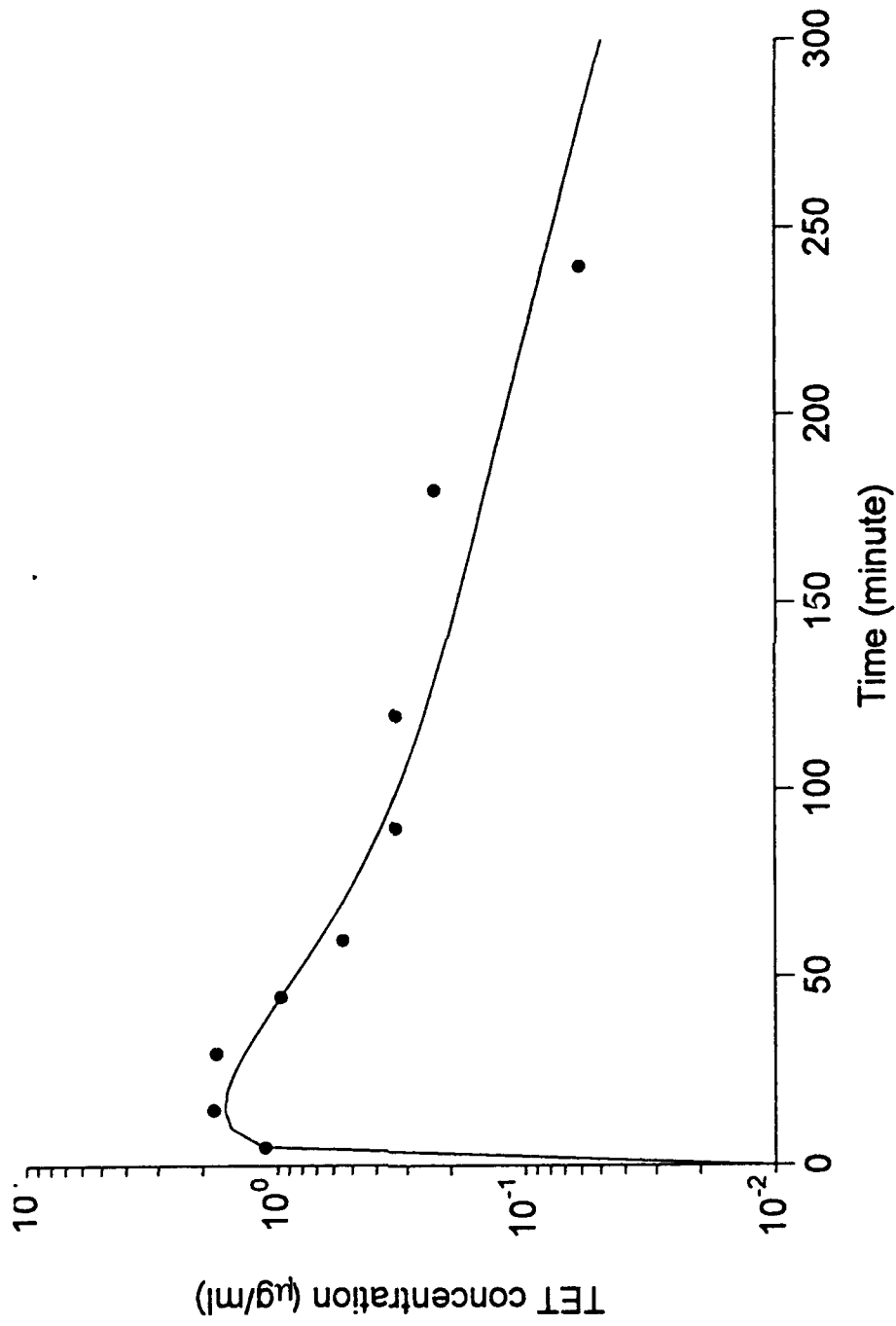
TET concentration in fat after oral administration in rat



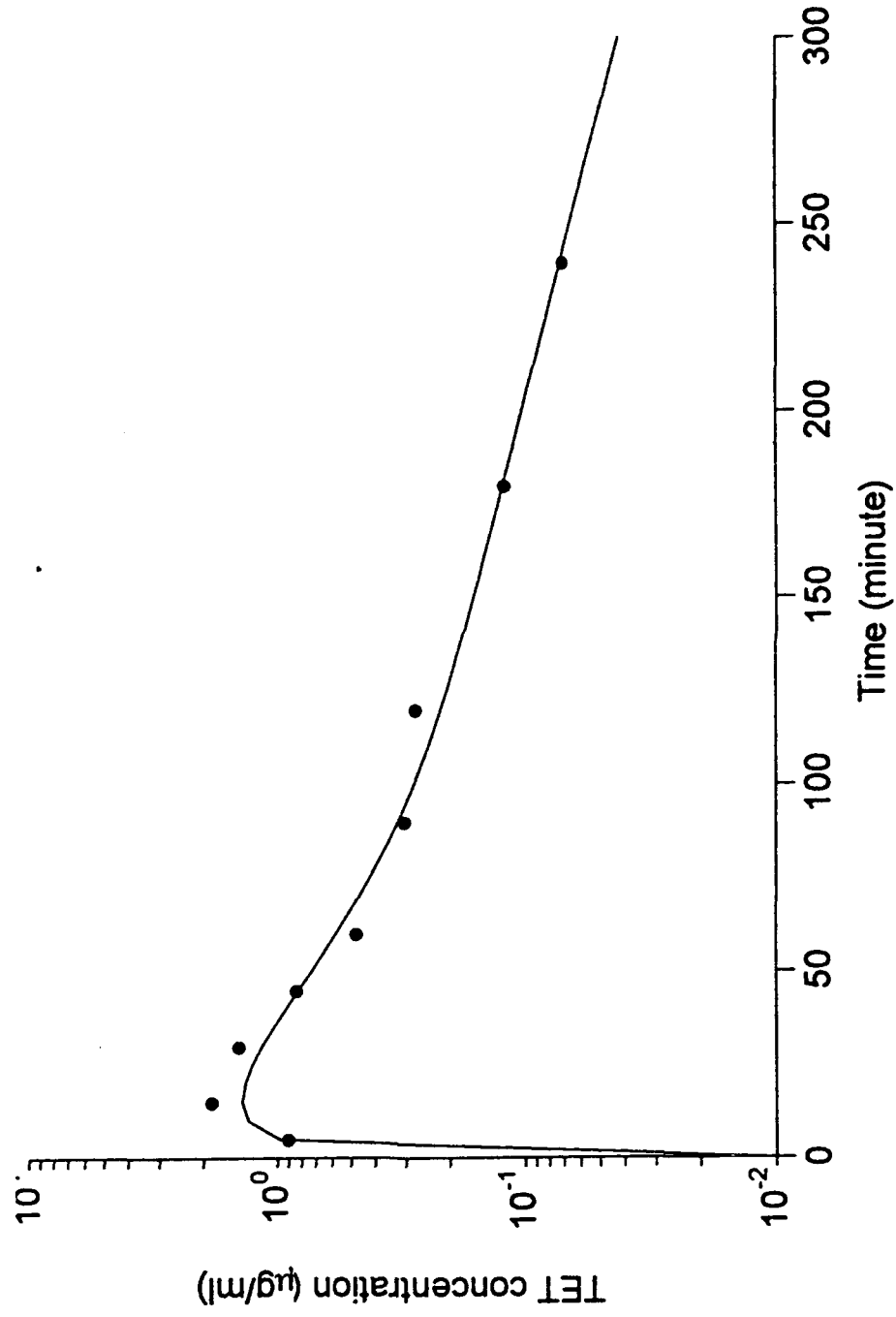
TET concentration in liver after oral administration in rat



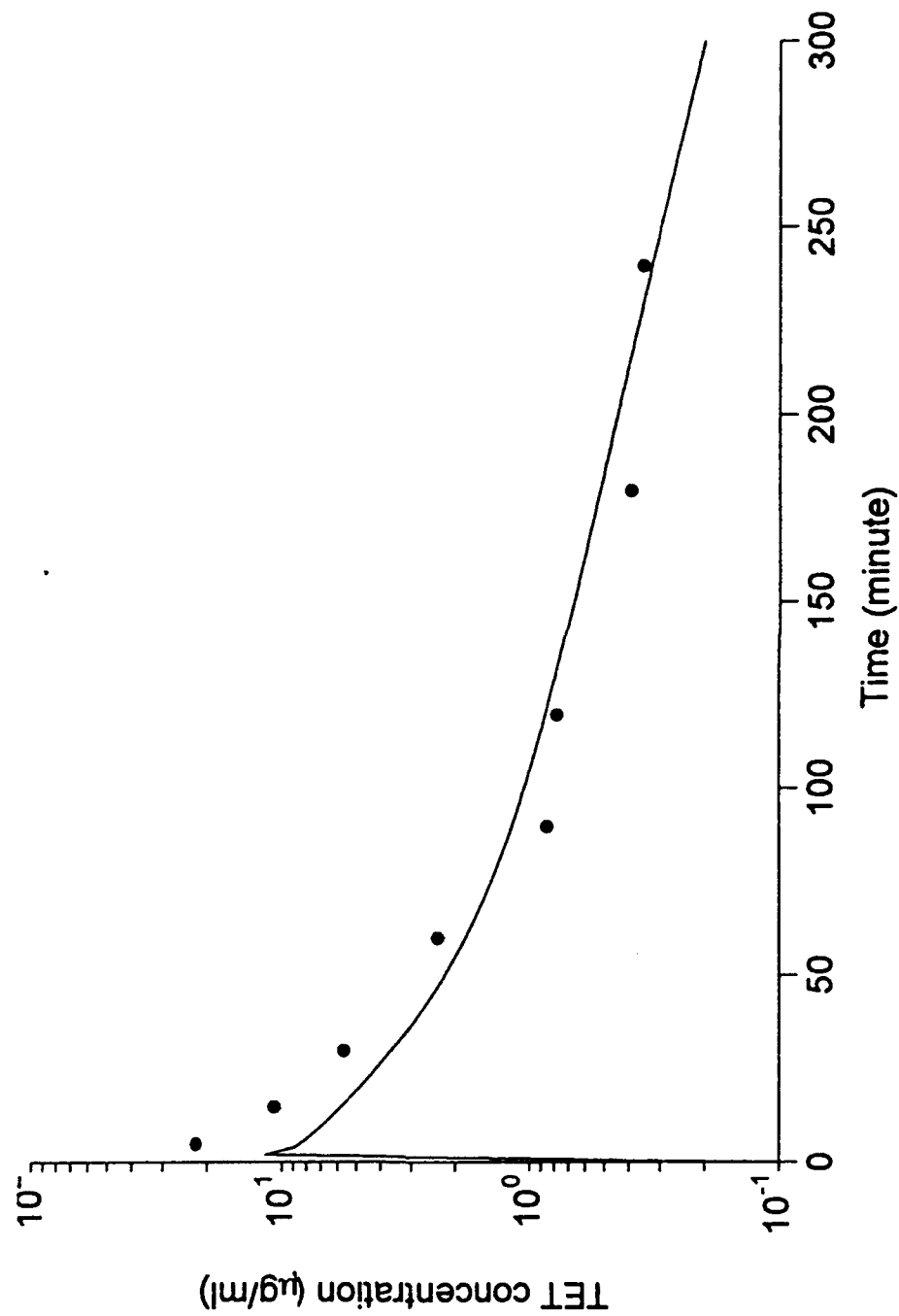
TET concentration in kidney after oral administration in rat



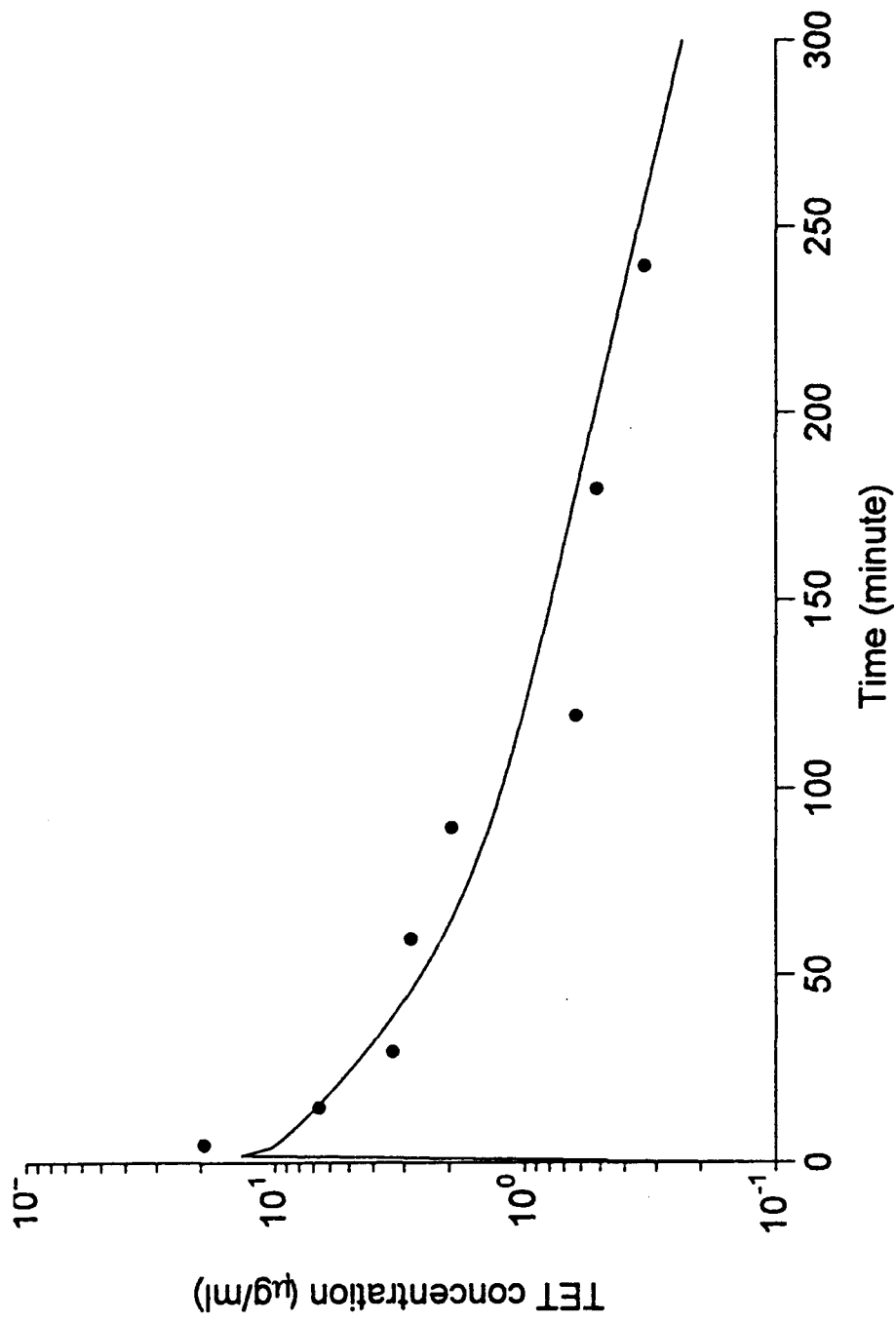
TET concentration in heart after oral administration in rat



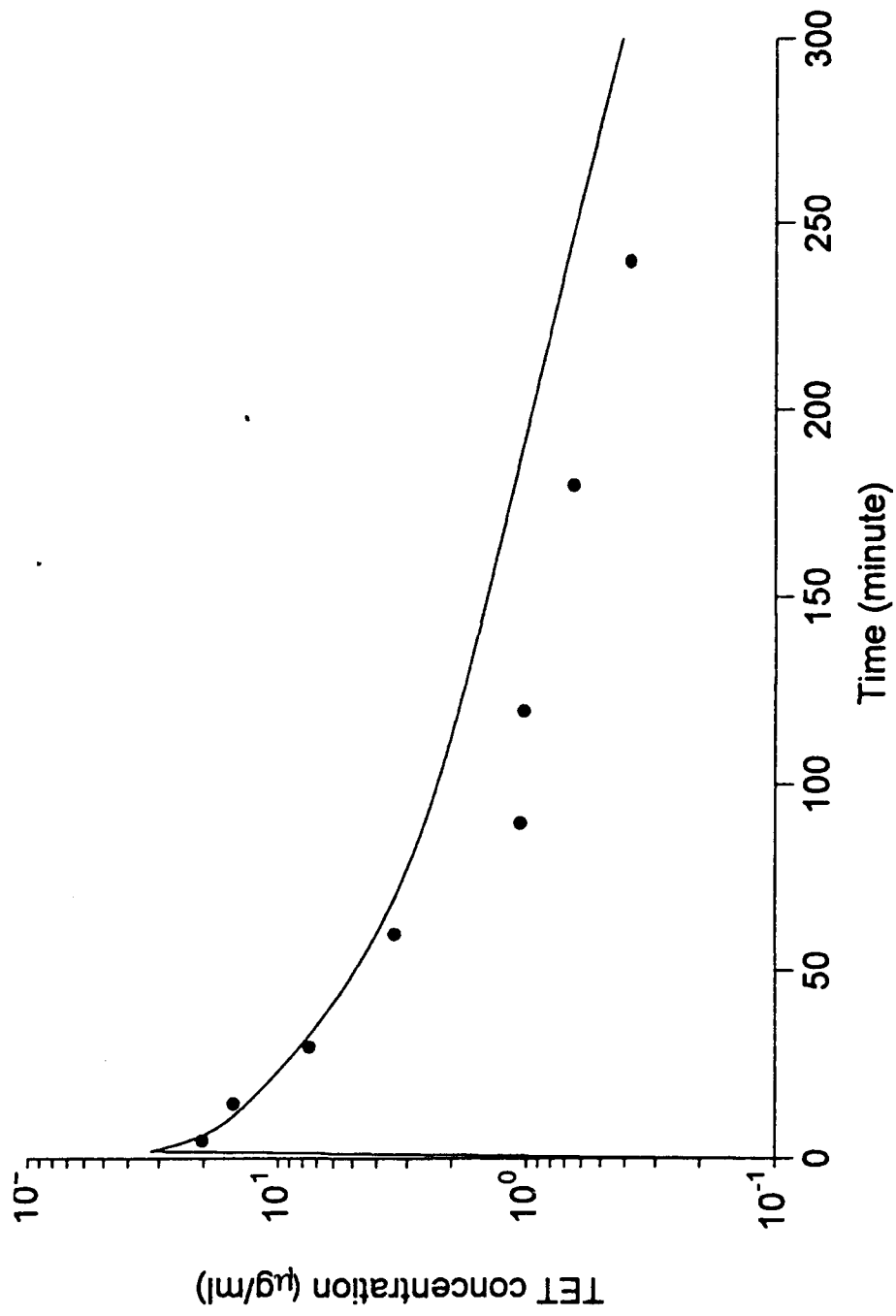
TET concentration in heart after i.a. administration in rat



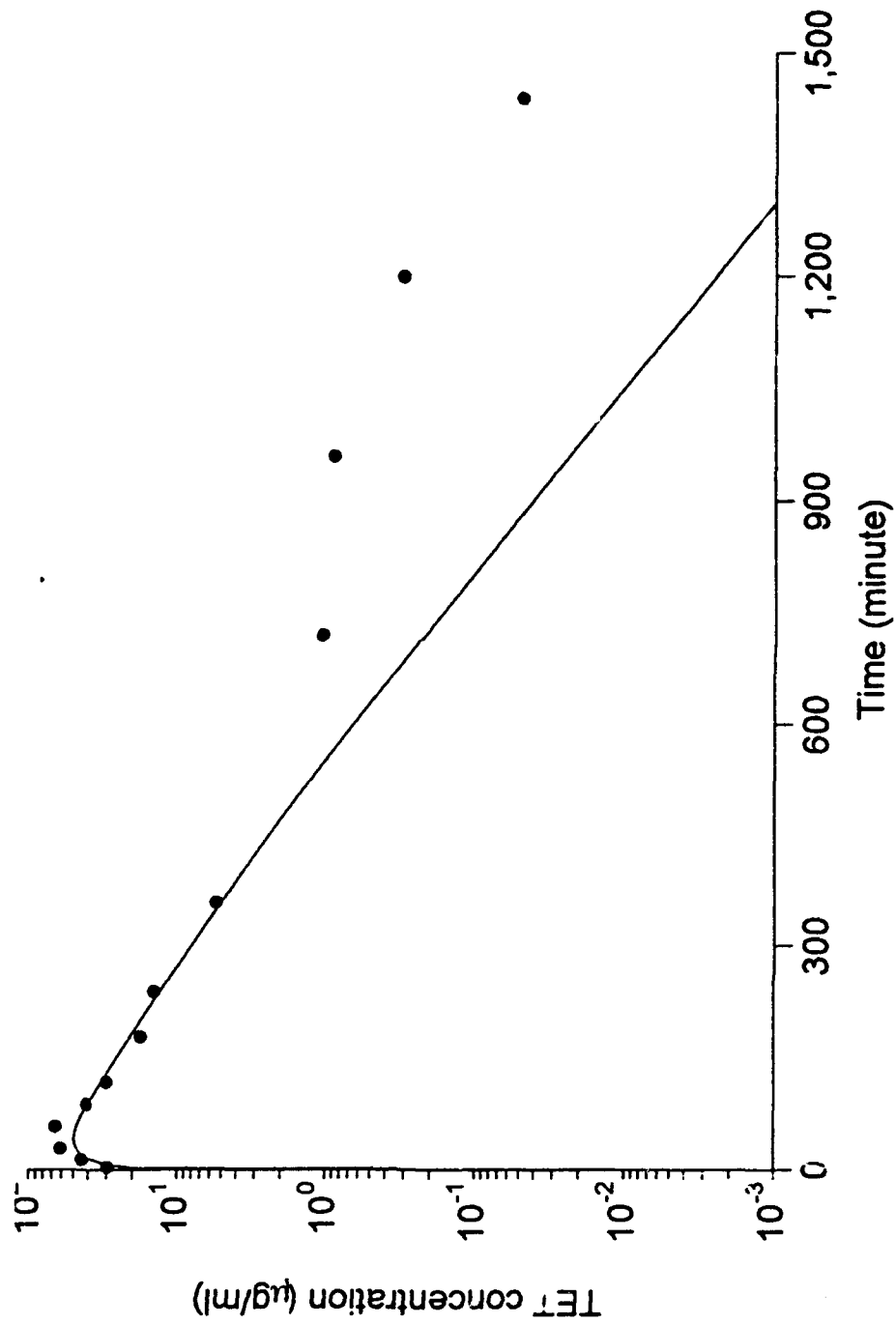
TET concentration in kidney after i.a. administration in rat



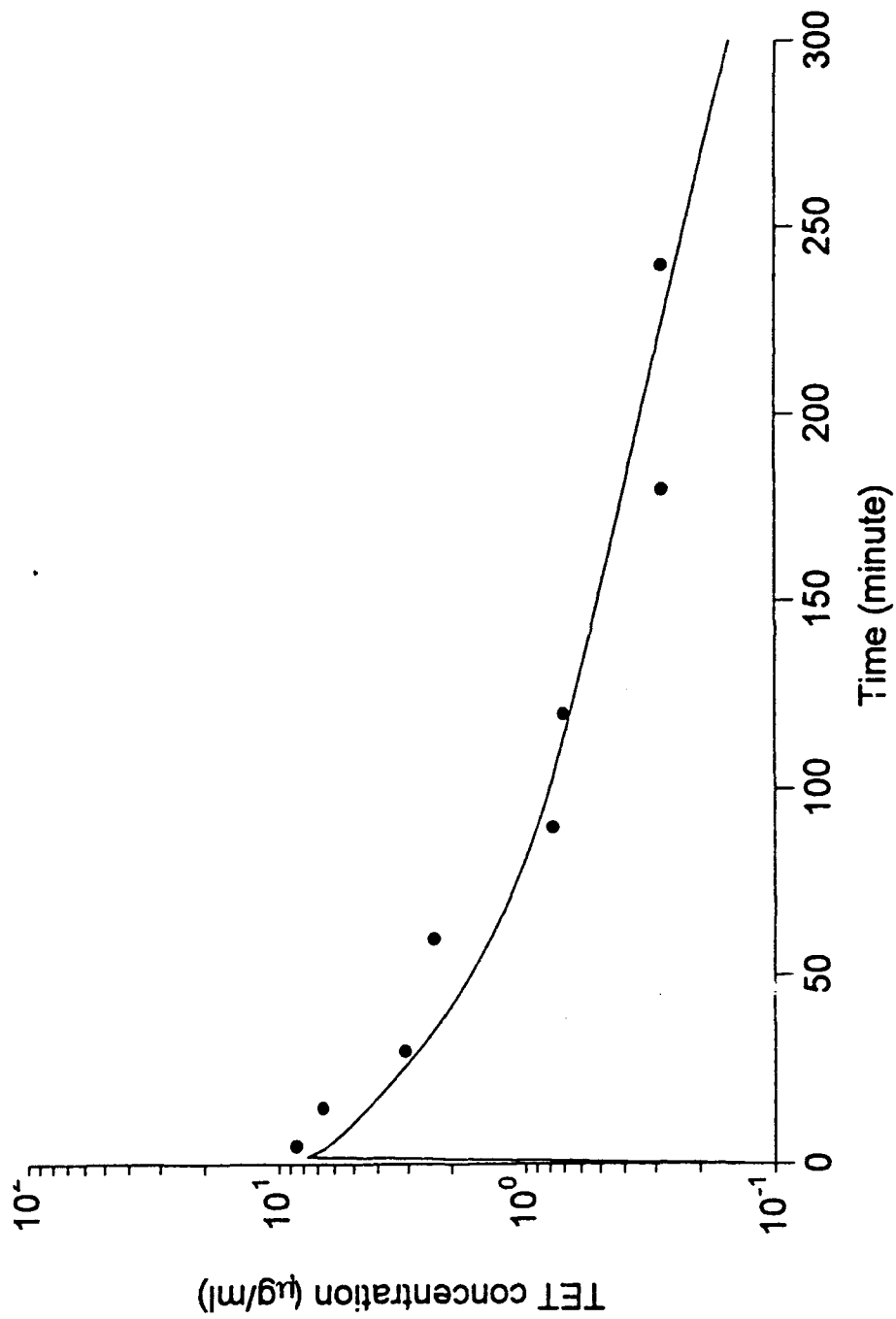
TET concentration in liver after i.a. administration in rat



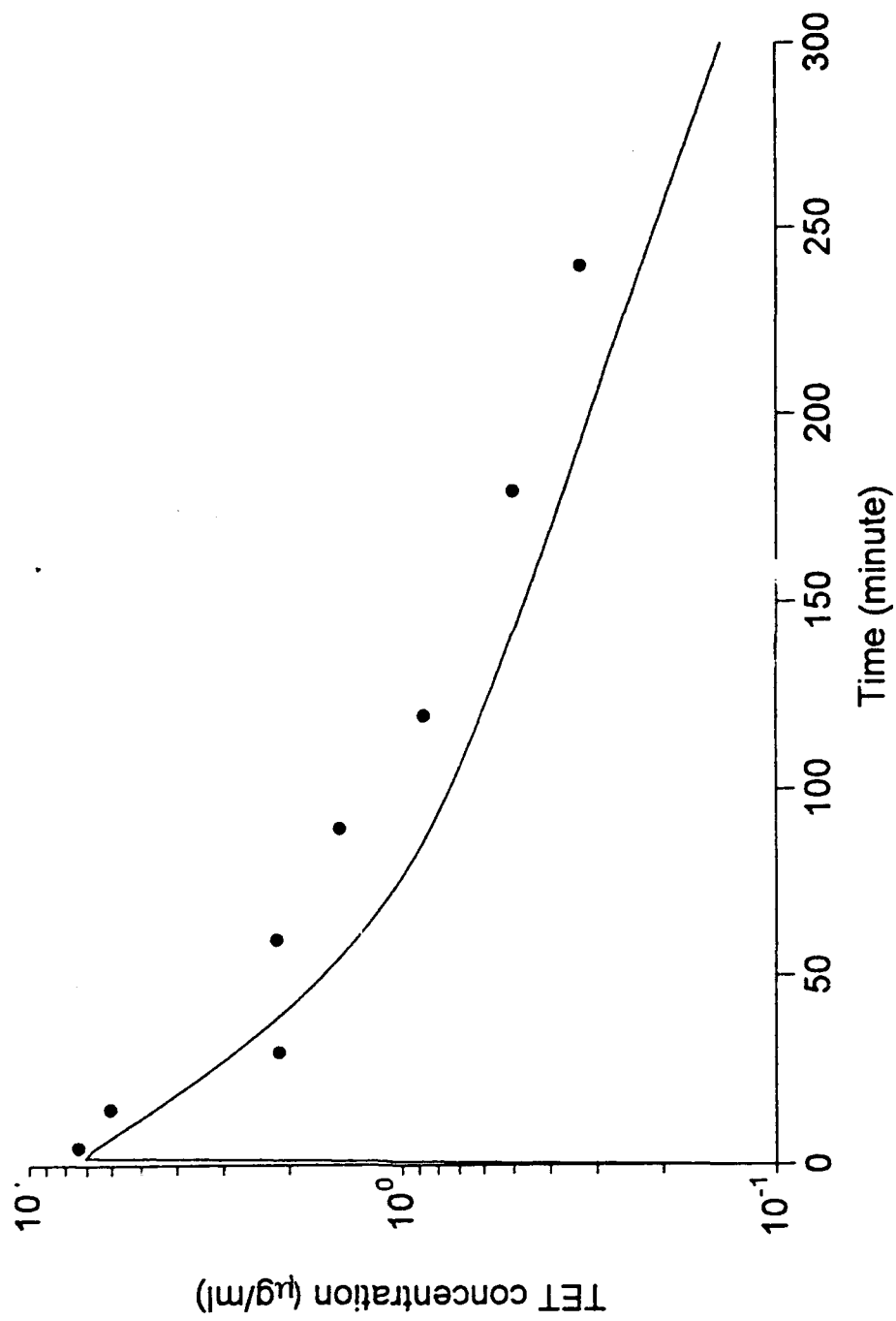
TET concentration in fat after i.a. administration in rat



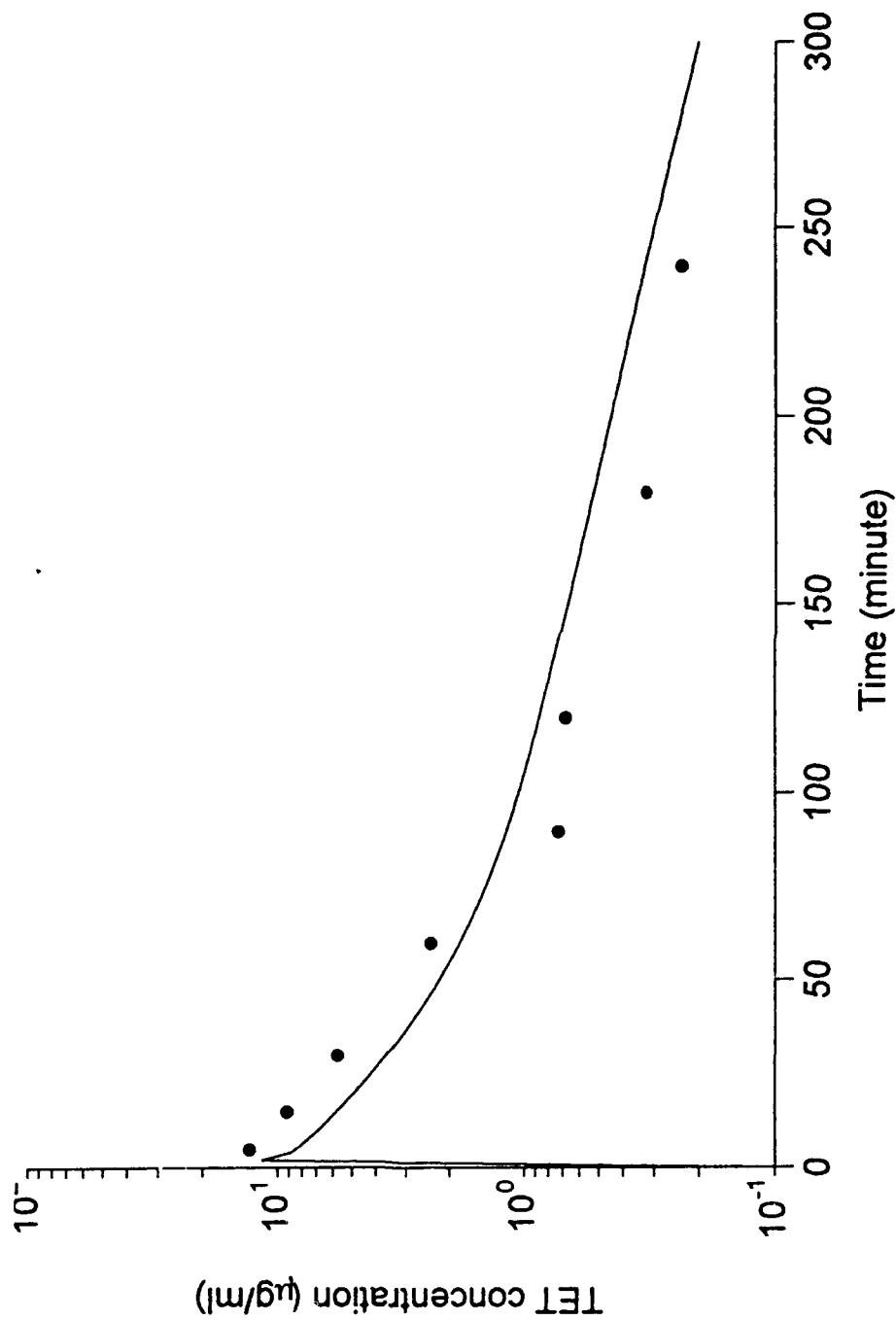
TET concentration in lung after i.a. administration in rat



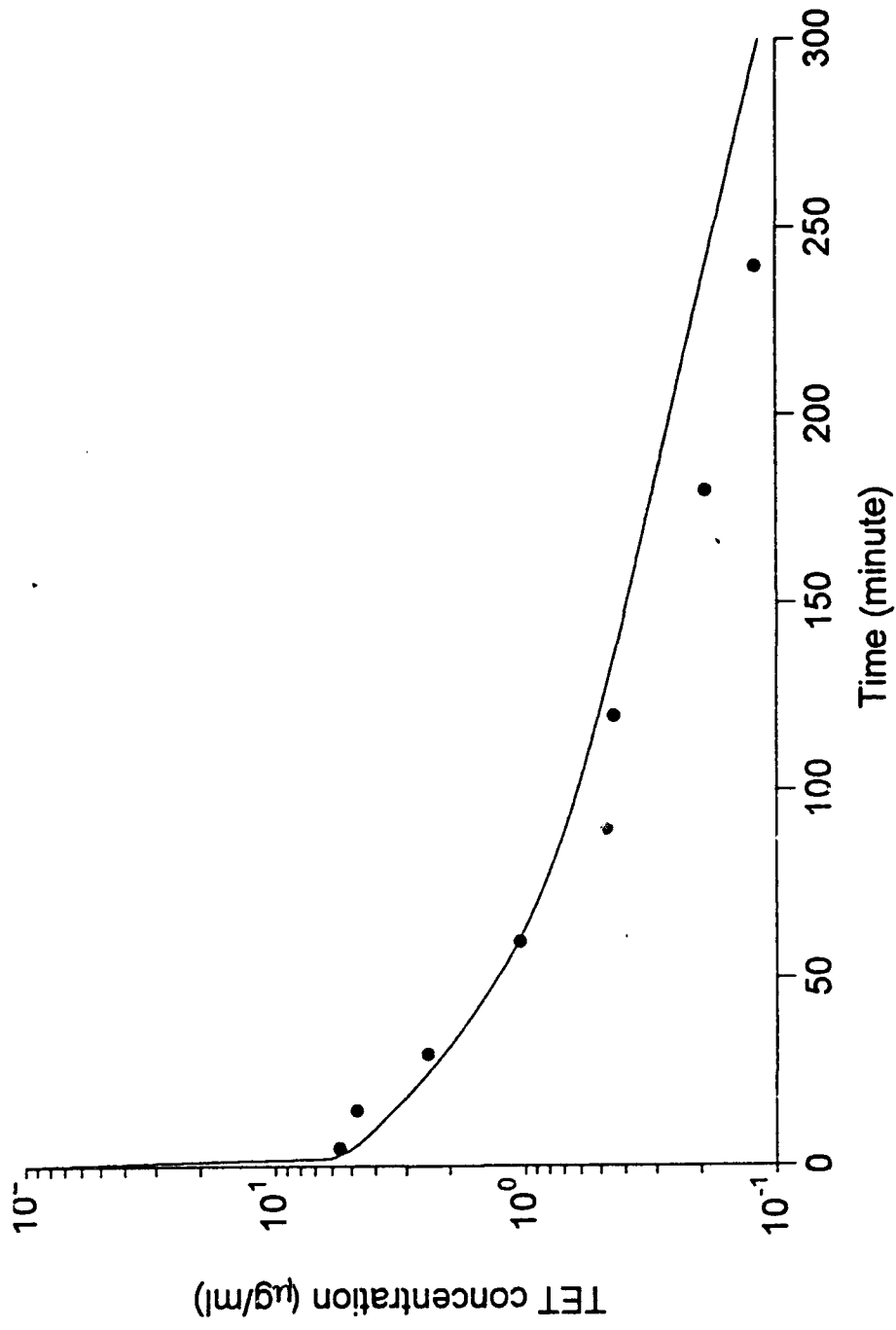
TET concentration in muscle after i.a. administration in rat



TET concentration in brain after i.a. administration in rat



TET concentration in arterial blood after i.a. administration in rat



APPENDIX

SECTION G

PBPK MODEL PREDICTIONS FOR TET IN THE
VENOUS BLOOD OF RATS AND DOGS

Parameters Used in the Physiologically Based
Pharmacokinetic Model for TET in the Rat and the Dog

Parameter	Rat	Dog
Tissue volumes (ml)		
liver	11.3	451.5
kidney	2.5	64.2
fat	6.6	1626.4
heart	1.09	120.9
lung	1.2	70.6
muscle	117.4	5007.6
brain	2.0	80.3
blood	24.6	876.3
rest of body	155.3	2402.1
Alveolar ventilation (ml/min)	124	2710
Blood flows (ml/min)		
liver	19.2	110.5
kidney	16.0	217.8
fat	8.0	108.4
heart	5.76	72.6
lung	100	100
muscle	31.8	875.3
brain	2.70	67.3
blood	121.8	2156.7
rest of body	38.4	32.68
Partition Coefficients		
Blood:air	7.5	12.3
Fat:blood	41.4	64.2
Lung:blood	1.81	1.60
Liver:blood	3.66	2.6
Muscle:blood	1.53	5.88
Brain:blood	2.09	2.77
Heart:blood	2.62	4.24
Kidney:blood	3.34	2.41
Rest of body:blood	1.53	5.88
Metabolism constants		
V _{max} (μg/min)	0.14	3.80
K _m (μg/ml)	0.017	1.24

TABLE 2

PHARMACOKINETIC PARAMETERS IN THE RAT¹

Dose (mg/kg)	Route ²	N	Area under Curve (μ g.min/ml)	Biological Half-Life (hrs)	Cmax (μ g/ml)	Tmax (min)	Bioavailability (F)	Clearance (ml.min/kg)
10	PO	9	61.49 \pm 5.83	1.02 \pm 0.13	1.23 \pm 0.20	12.67 \pm 1.69	0.35	174.15 \pm 15.6
10	IA	8	174.50 \pm 16.57	1.41 \pm 0.04	3.95 \pm 0.55	6.25 \pm 0.59		61.11 \pm 5.79
30	PO	5	611.99 \pm 71.34	2.60 \pm 0.62	6.28 \pm 1.16	13.00 \pm 1.24	0.51	52.04 \pm 6.55
30	IA	7	1202.64 \pm 169.01	2.31 \pm 0.16	19.10 \pm 1.4	5.43 \pm 1.04		28.38 \pm 4.23

¹ Each value represents the mean \pm s.e.² PO is oral administration, and IA is intraarterial administration.

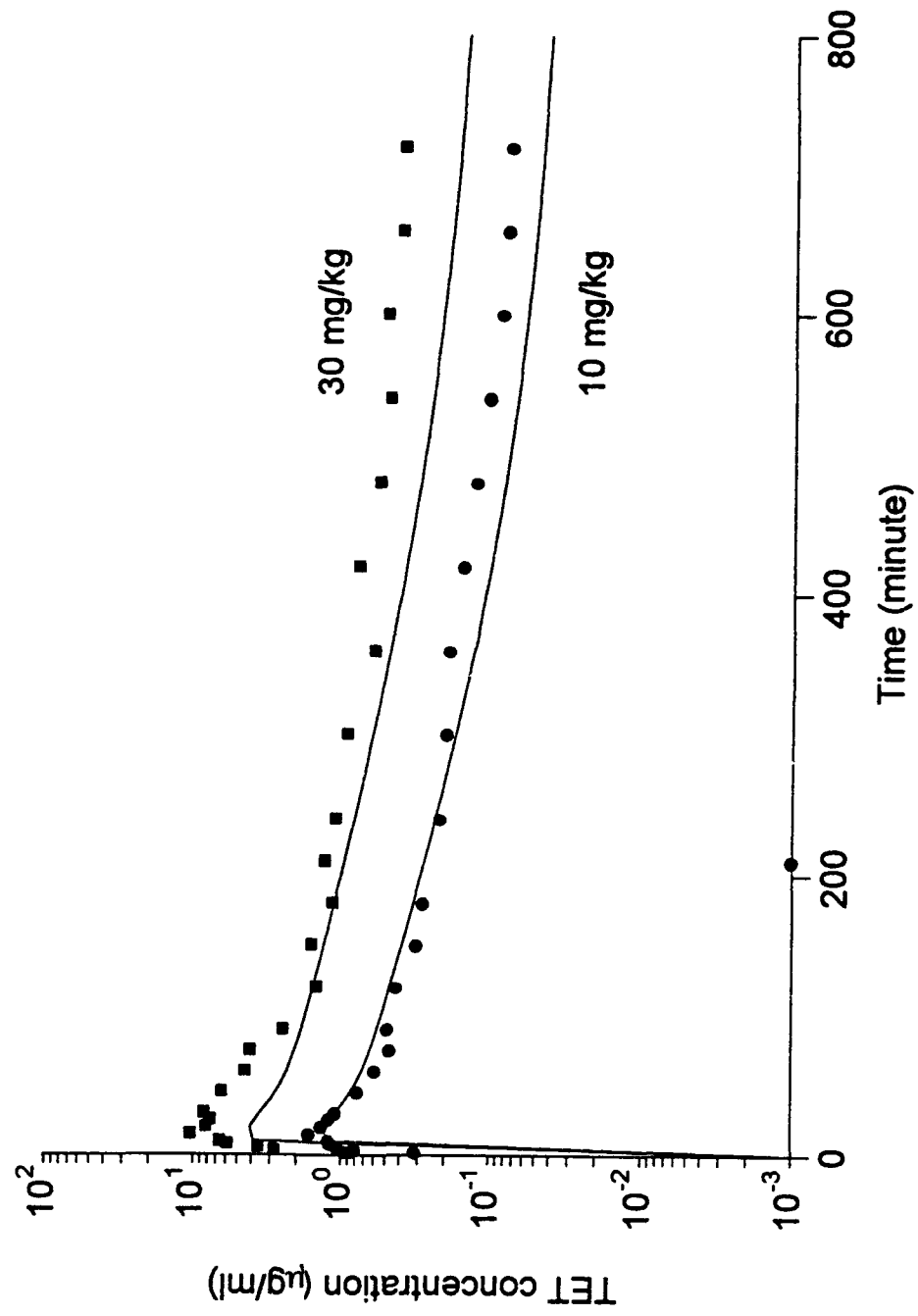
TABLE 3

PHARMACOKINETIC PARAMETERS IN THE DOG¹

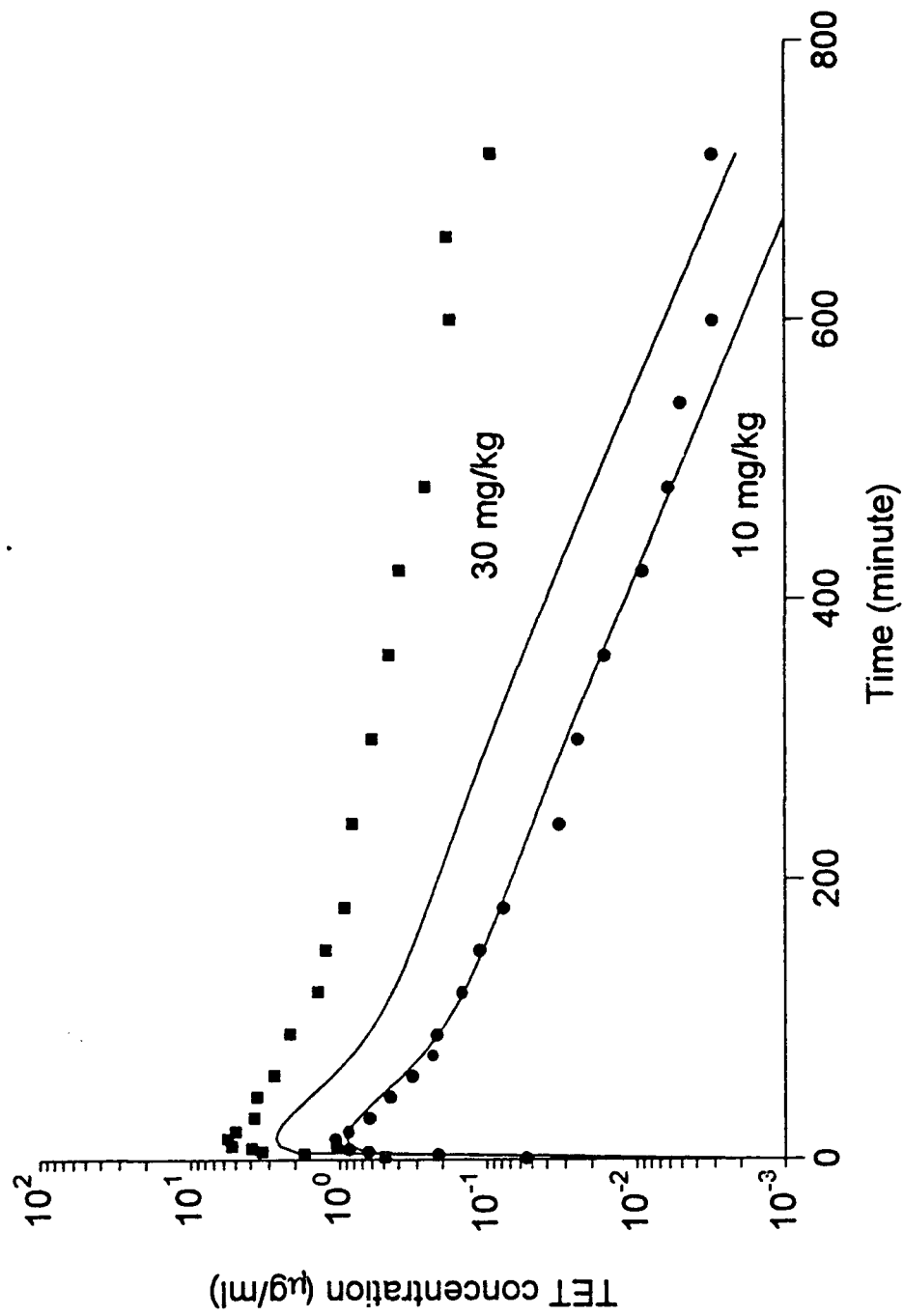
Dose (mg/kg)	Route ²	N	Area Under Curve (μ g.min/ml)	Biological Half-Life (hrs)	Cmax (μ g/ml)	Tmax (min)	Bioavailability (F)	Clearance (ml.min/kg)
10	PO	4	224.02 \pm 39.64	7.41 \pm 0.42	1.95 \pm 0.62	14.75 \pm 4.29	0.69	49.01 \pm 8.30
10	IA	4	326.13 \pm 33.05	9.52 \pm 1.06	9.09 \pm 1.39	2.00 \pm 0.71		31.70 \pm 3.46
30	PO	4	1207.52 \pm 282.28	8.56 \pm 1.01	9.93 \pm 2.26	21.25 \pm 5.14		29.45 \pm 6.36

¹ Each value represents the mean \pm s.e.² PO is oral administration, and IA is intraarterial administration.

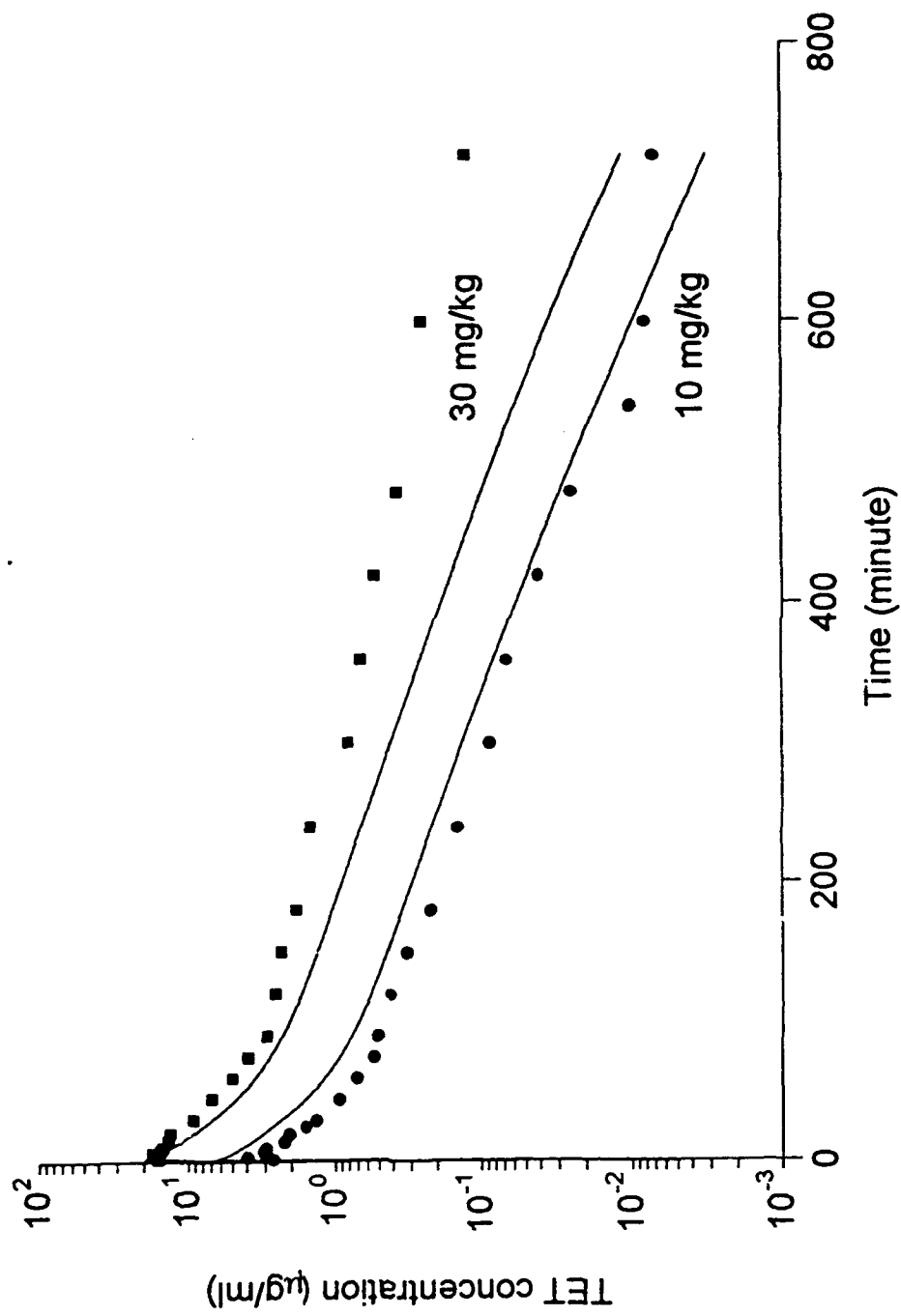
TET concentration in venous blood after oral administration in dog



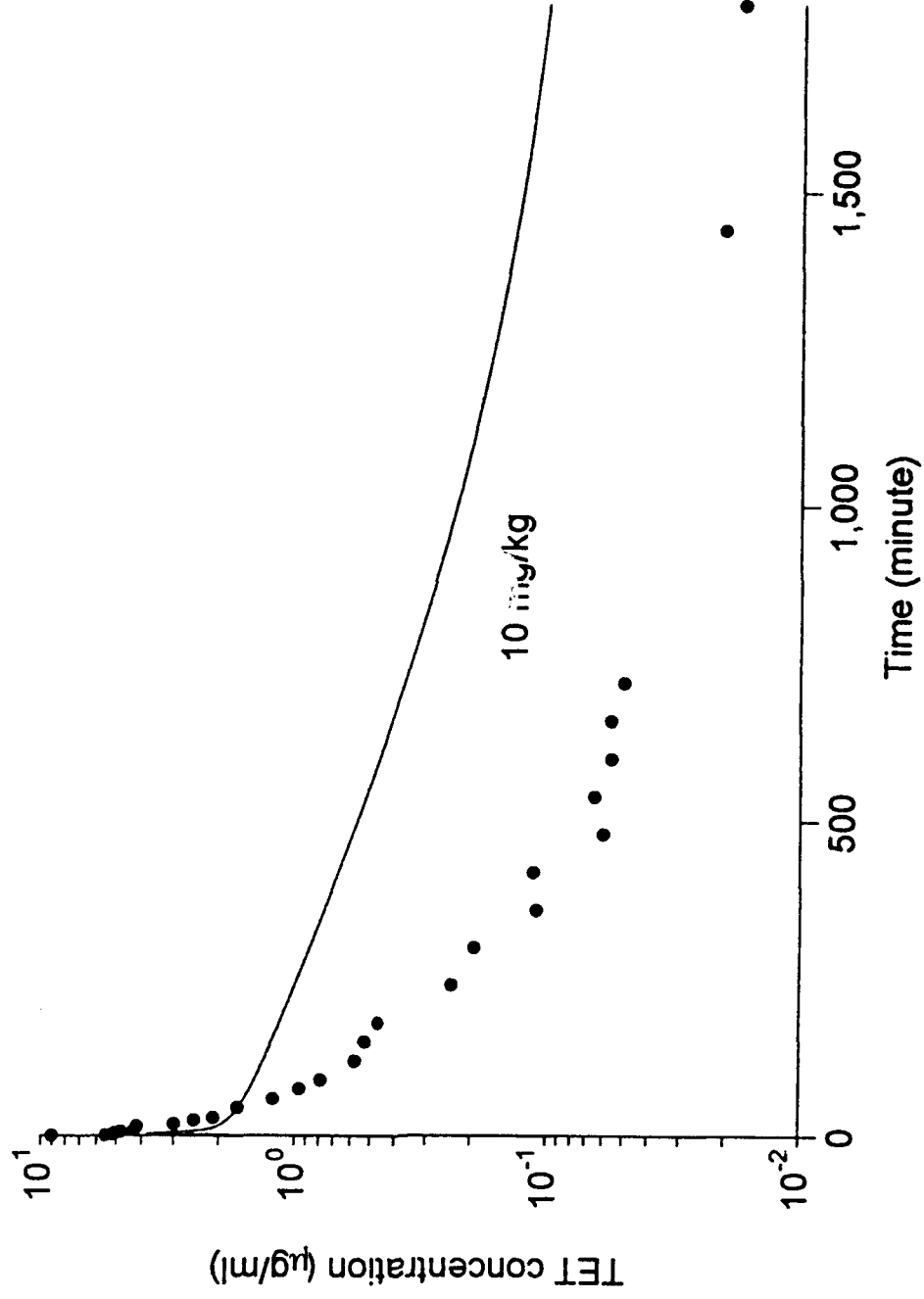
TET concentration in venous blood after oral administration in rat



TET concentration in venous blood after i.a. administration in rat



TET concentration in venous blood after i.a. administration in dog

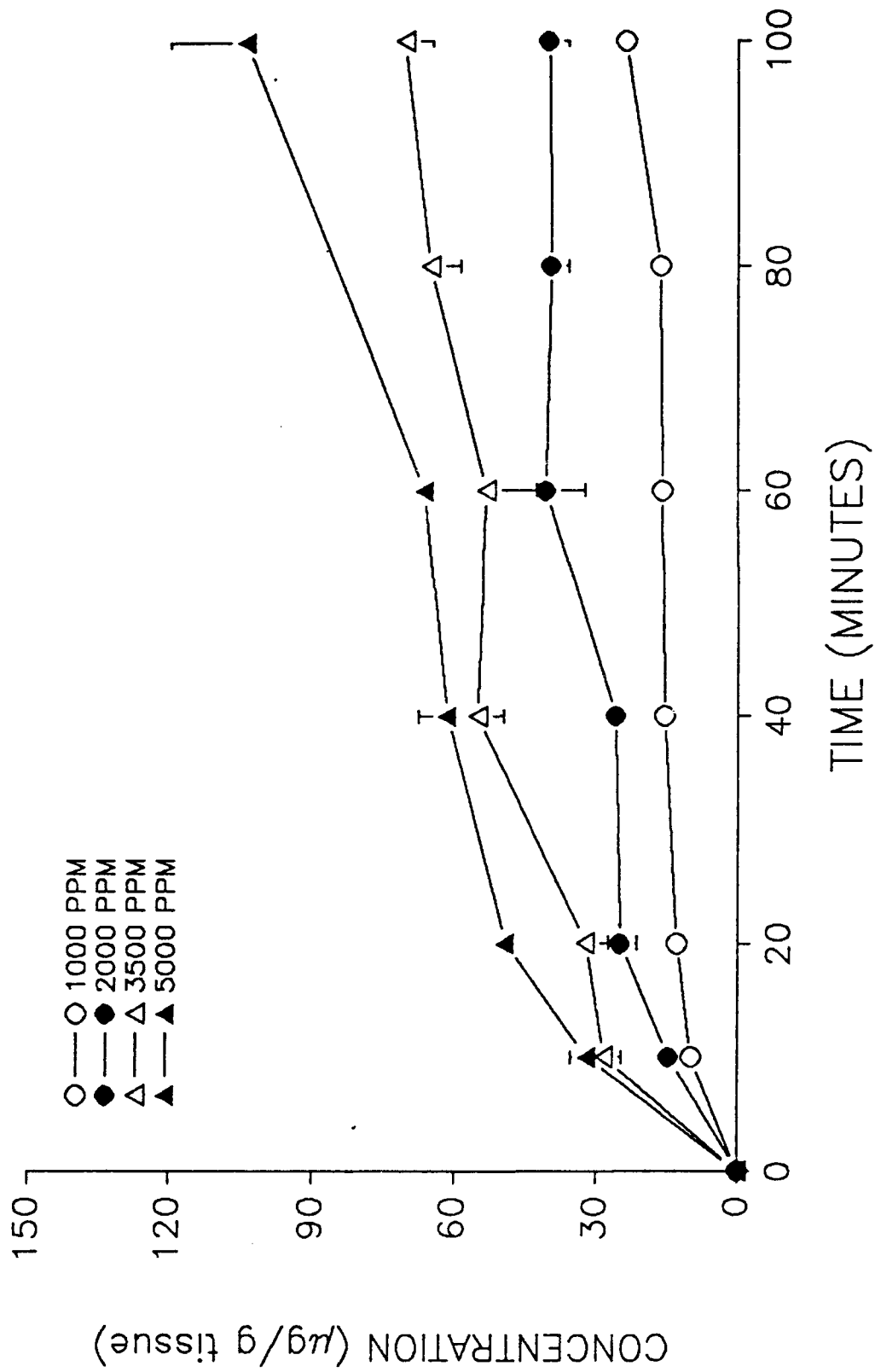


APPENDIX

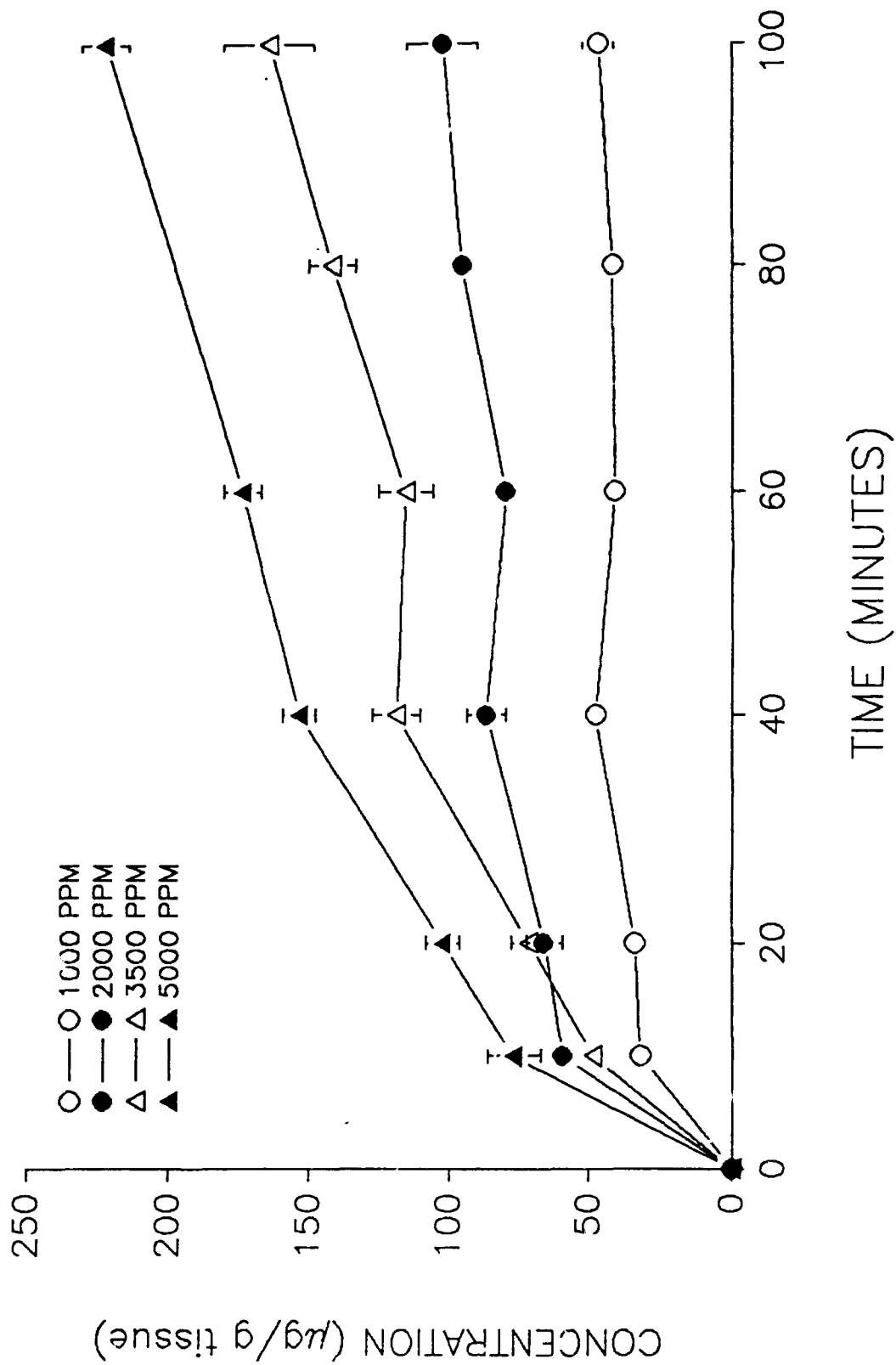
SECTION H

COMPARISON OF NEUROBEHAVIORAL EFFECTS OF TRI OVER TIME
WITH BRAIN DISPOSITION DURING INHALATION EXPOSURE

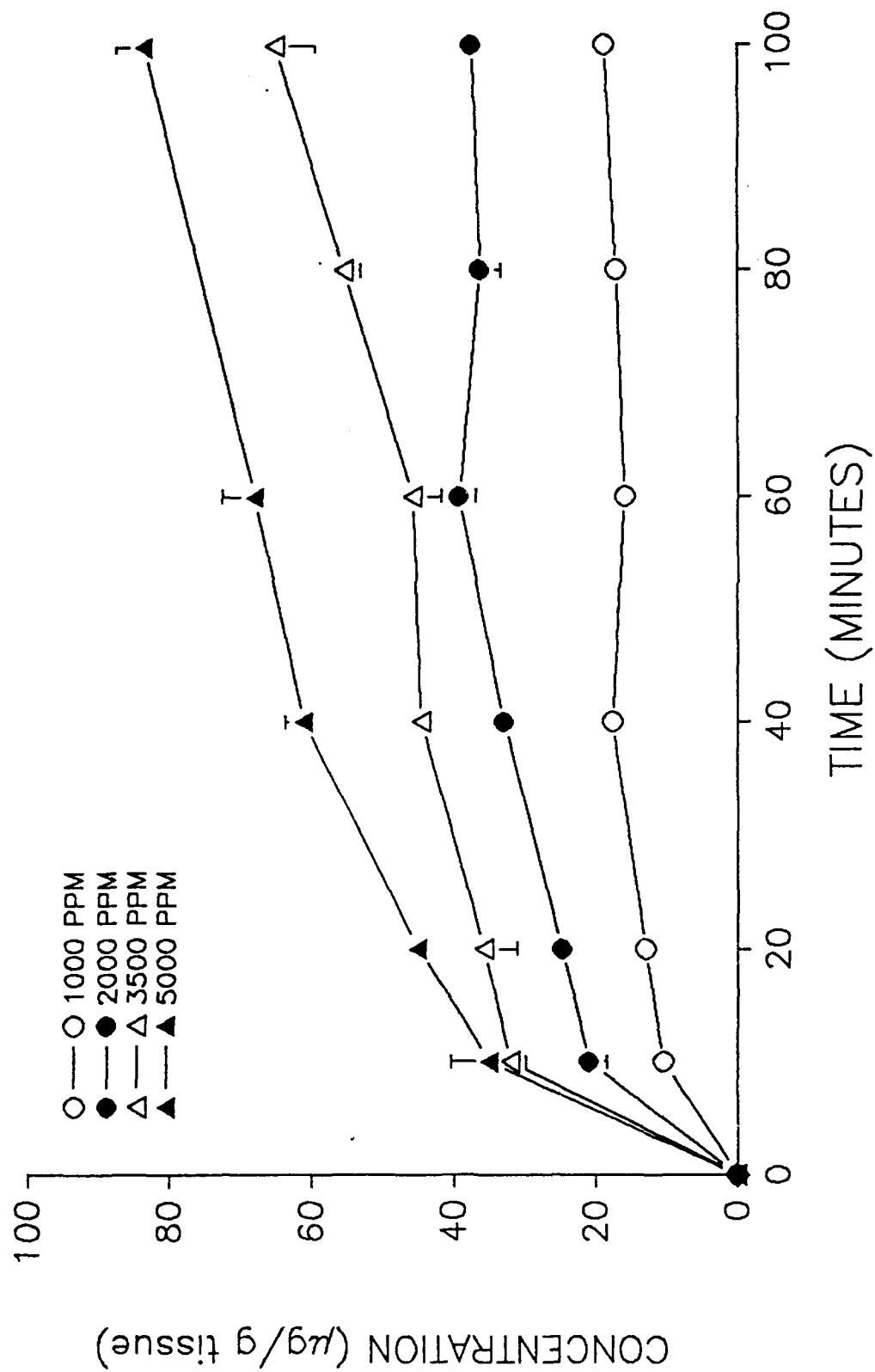
LUNG



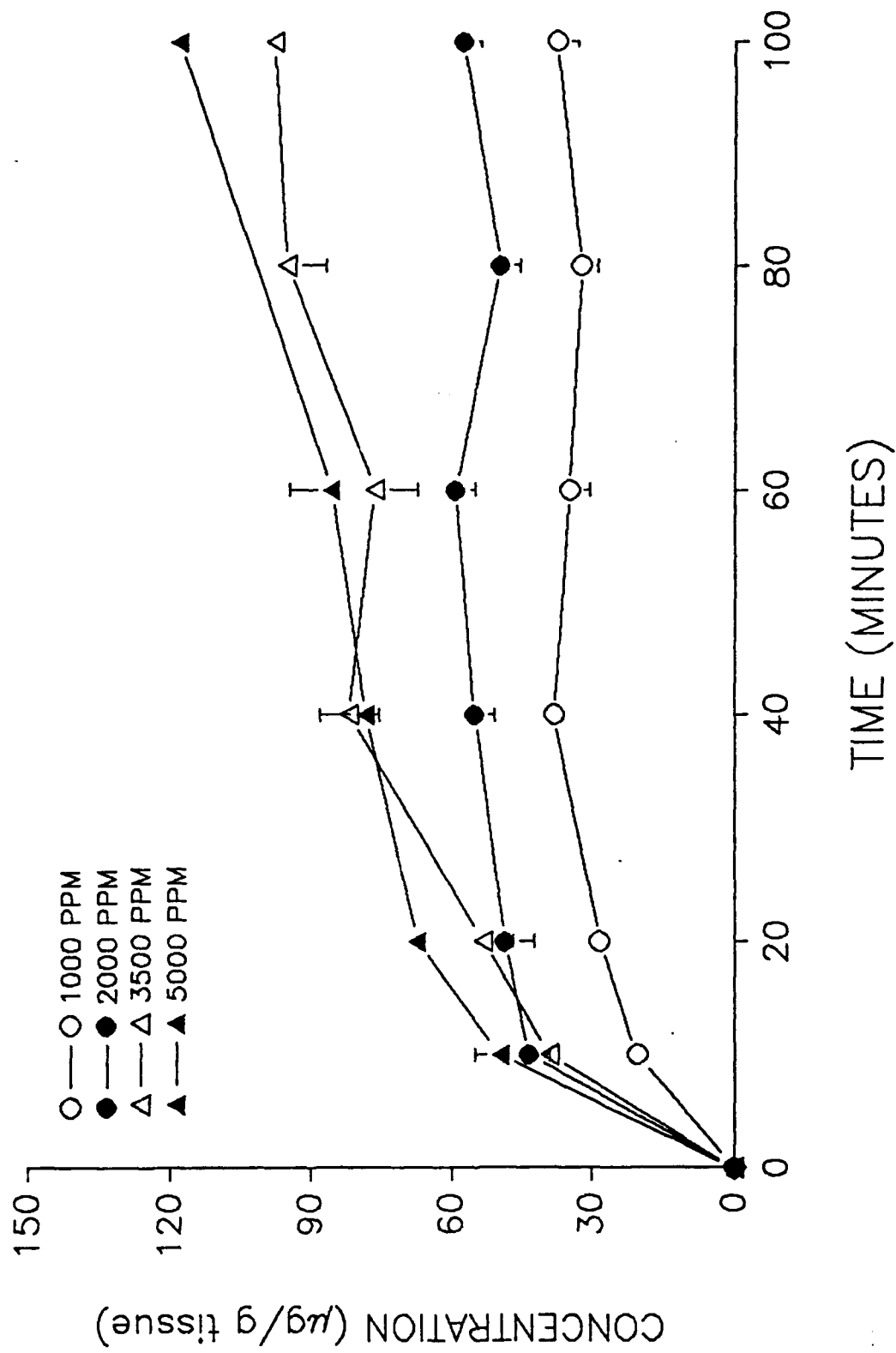
LIVER



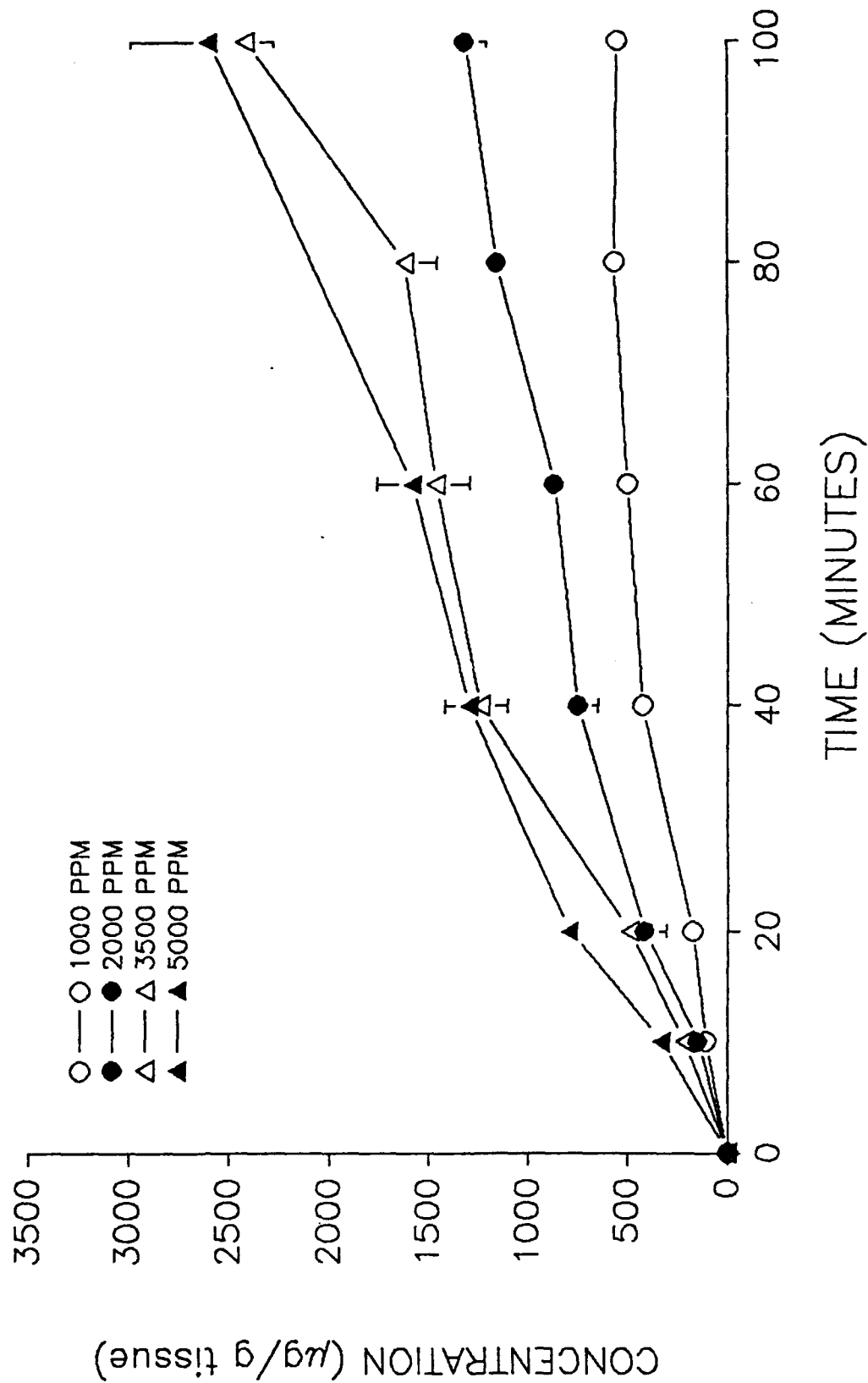
SPLEEN



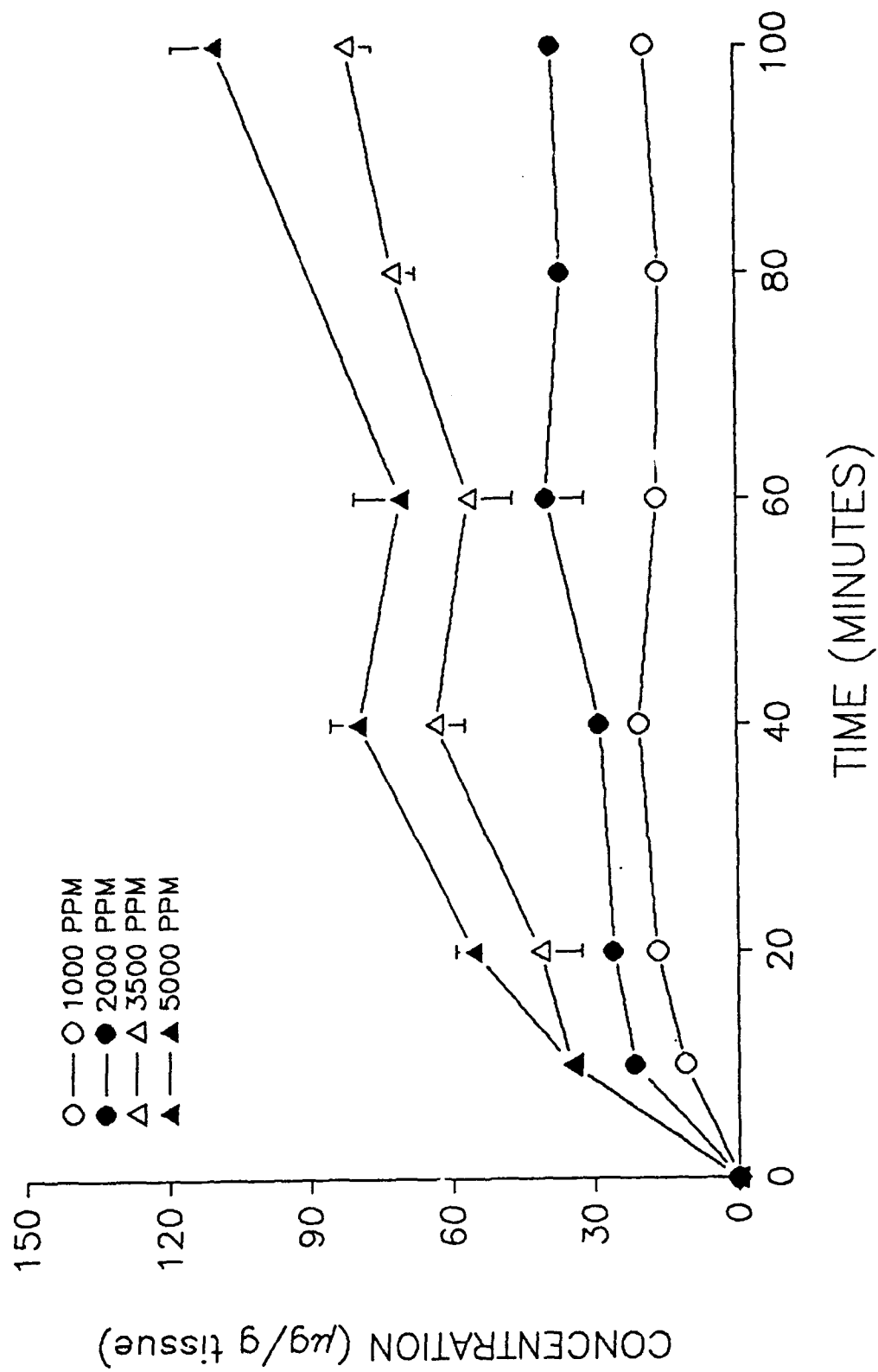
KIDNEY



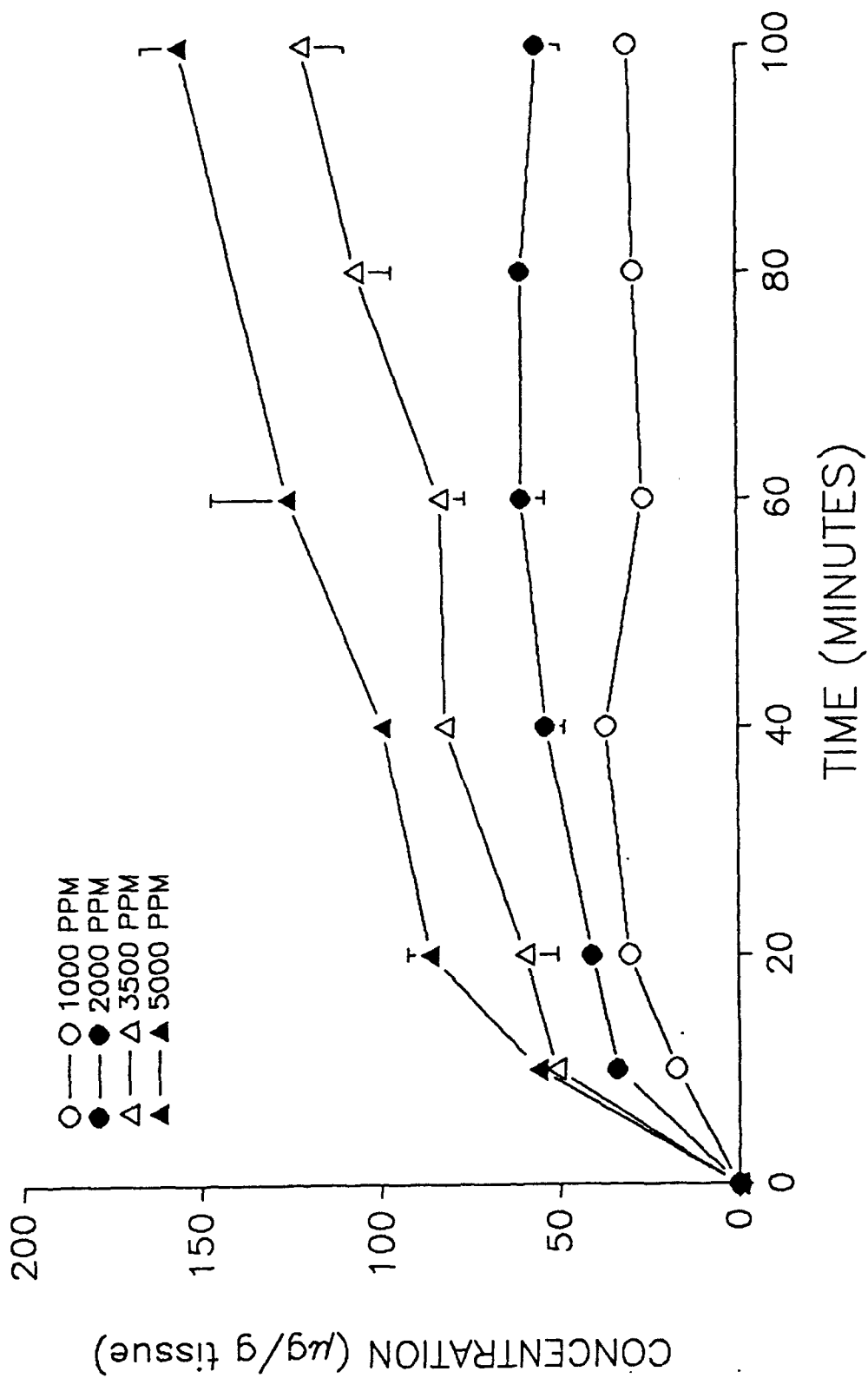
FAT



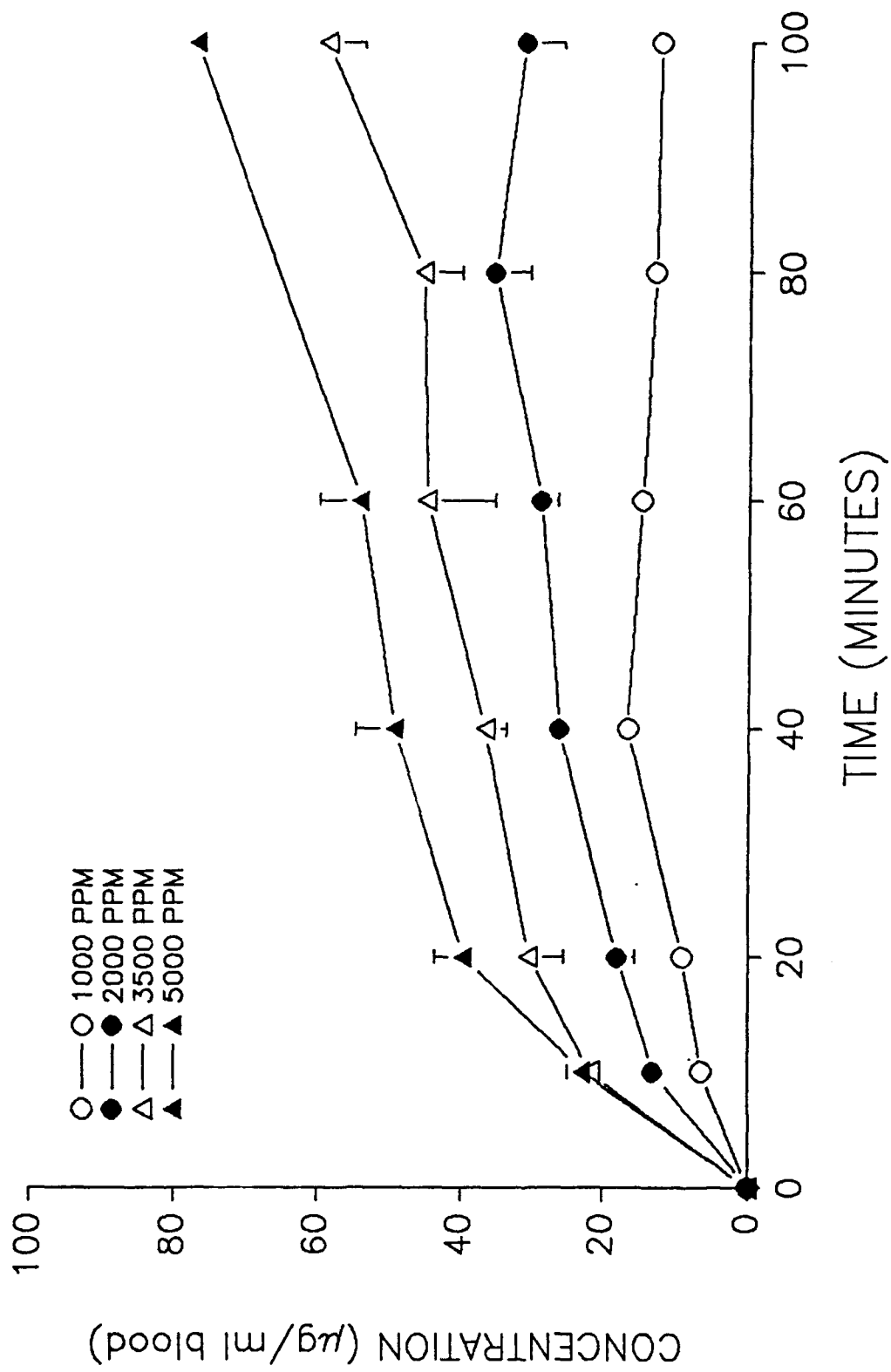
HEART



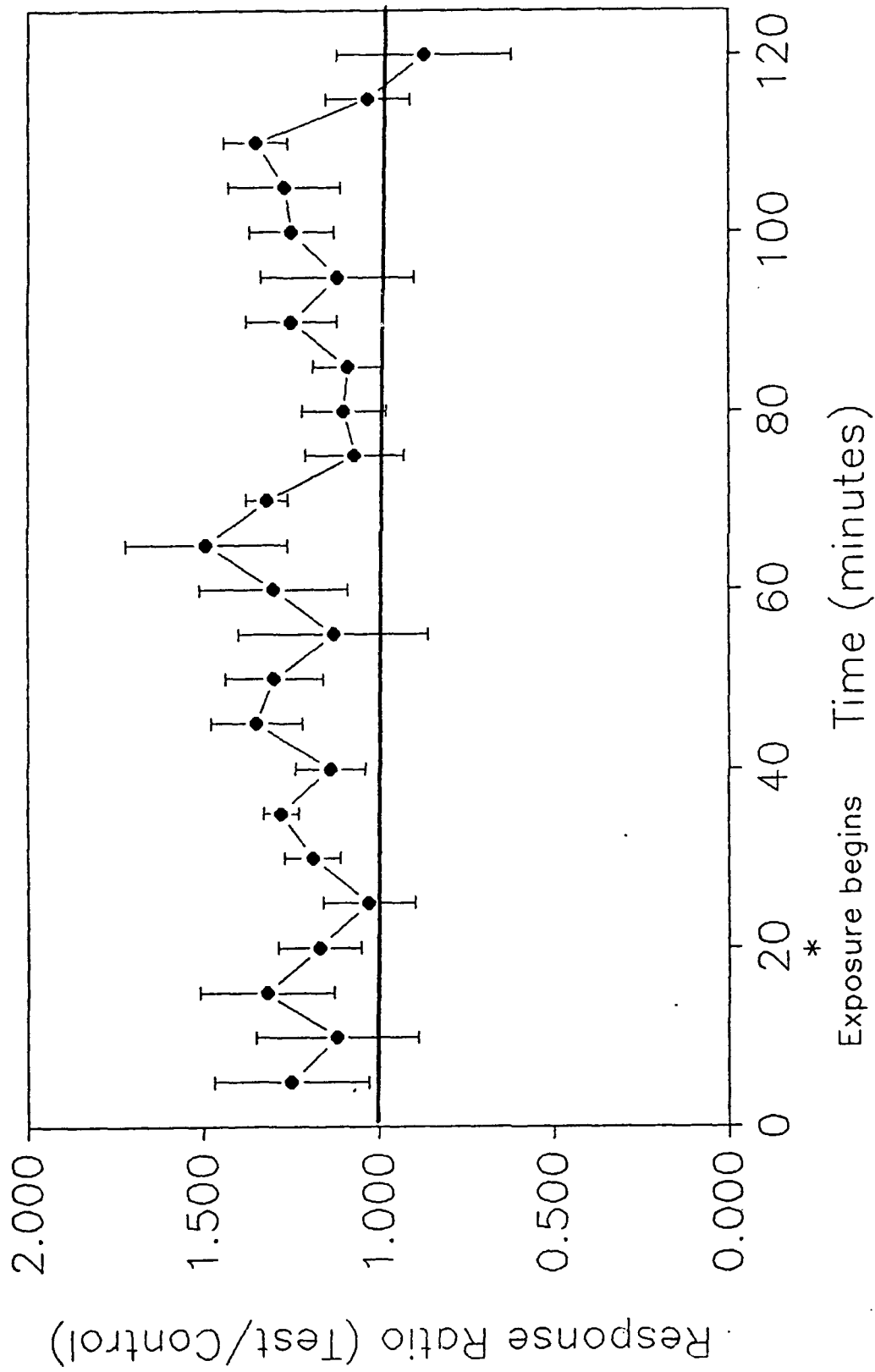
BRAIN



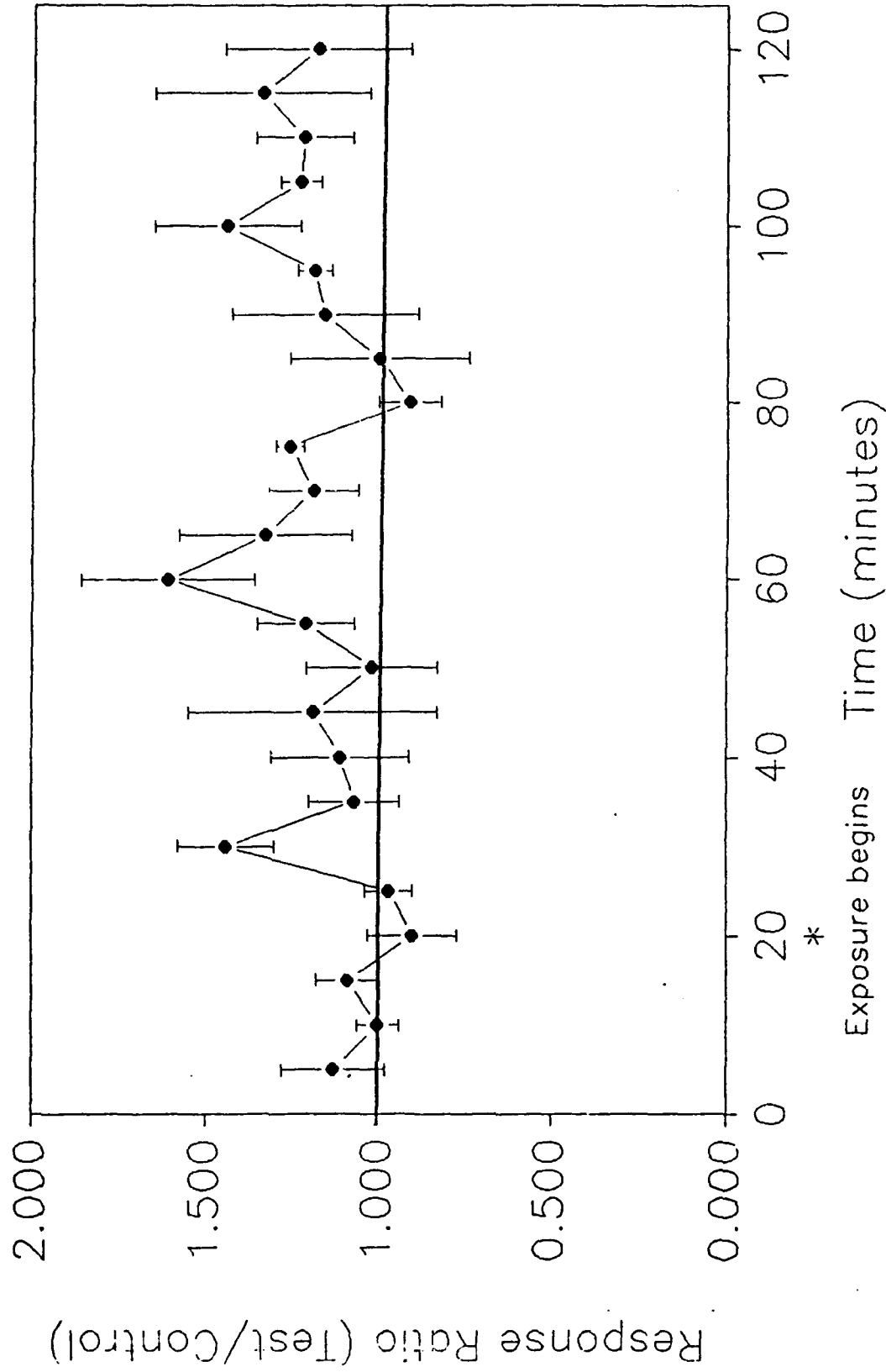
BLOOD



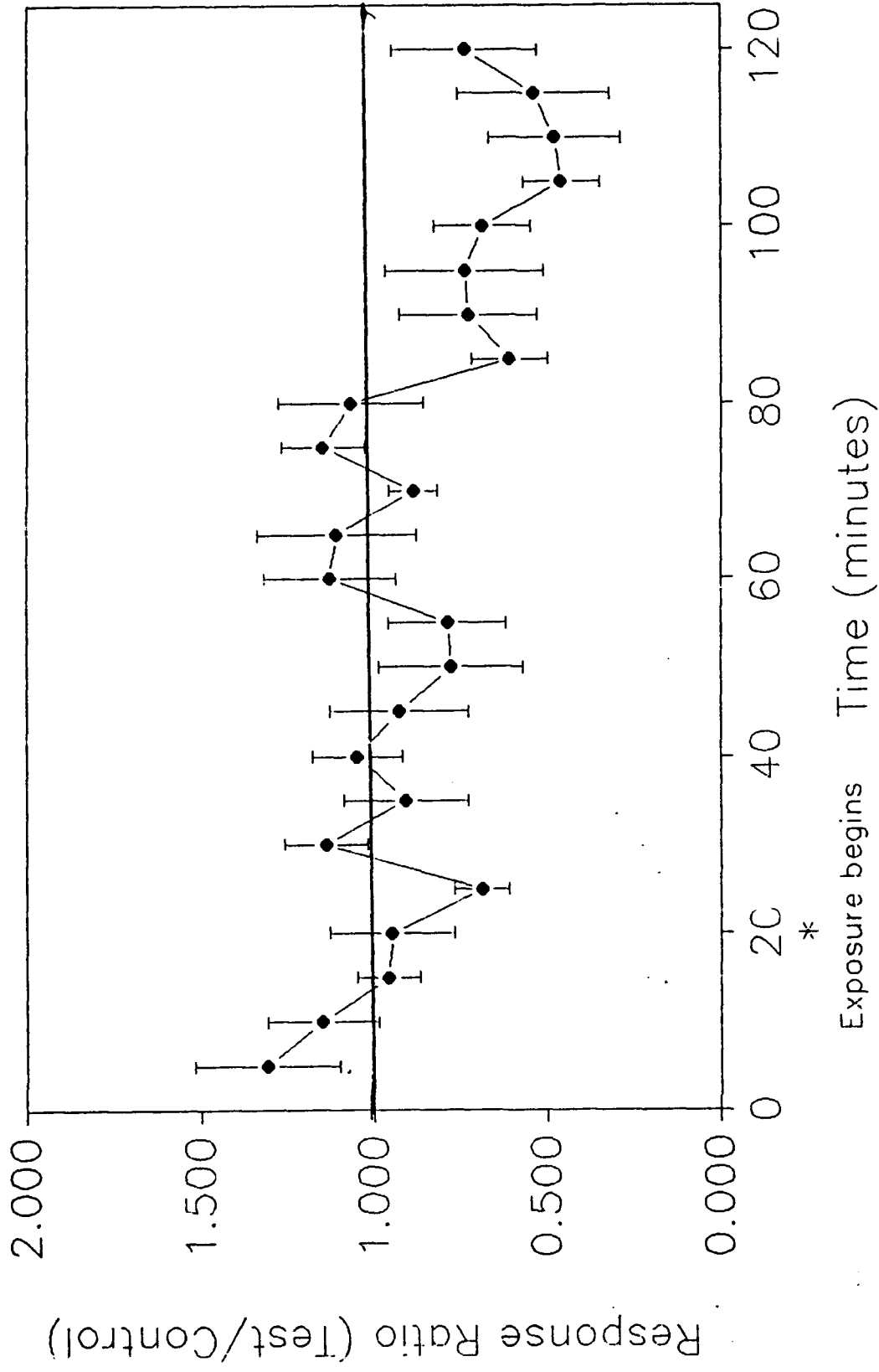
Responses During Inhalation of 500 ppm TRI



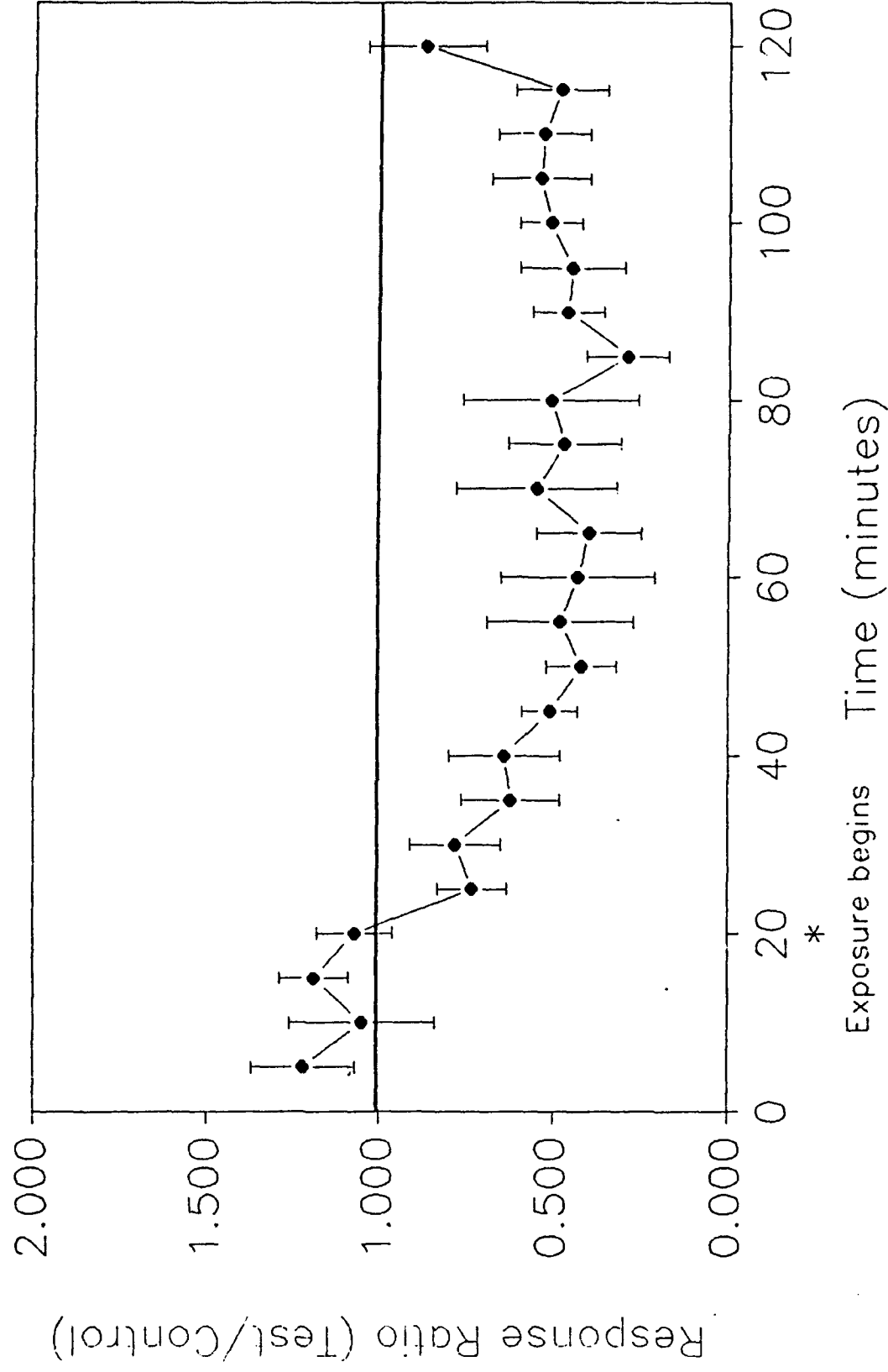
Responses During Inhalation of 1000 ppm TRI



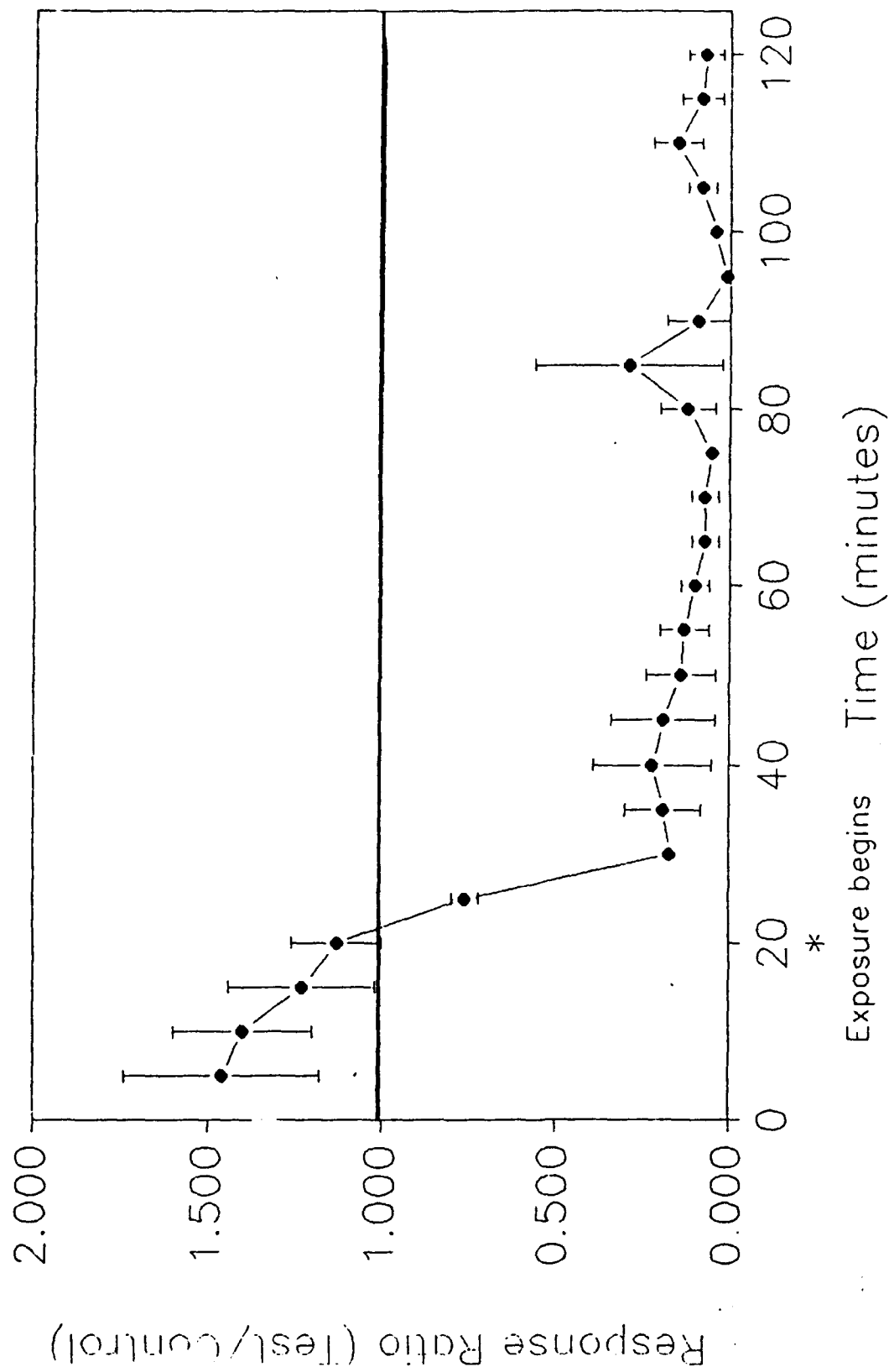
Responses During Inhalation of 2000 ppm TRI



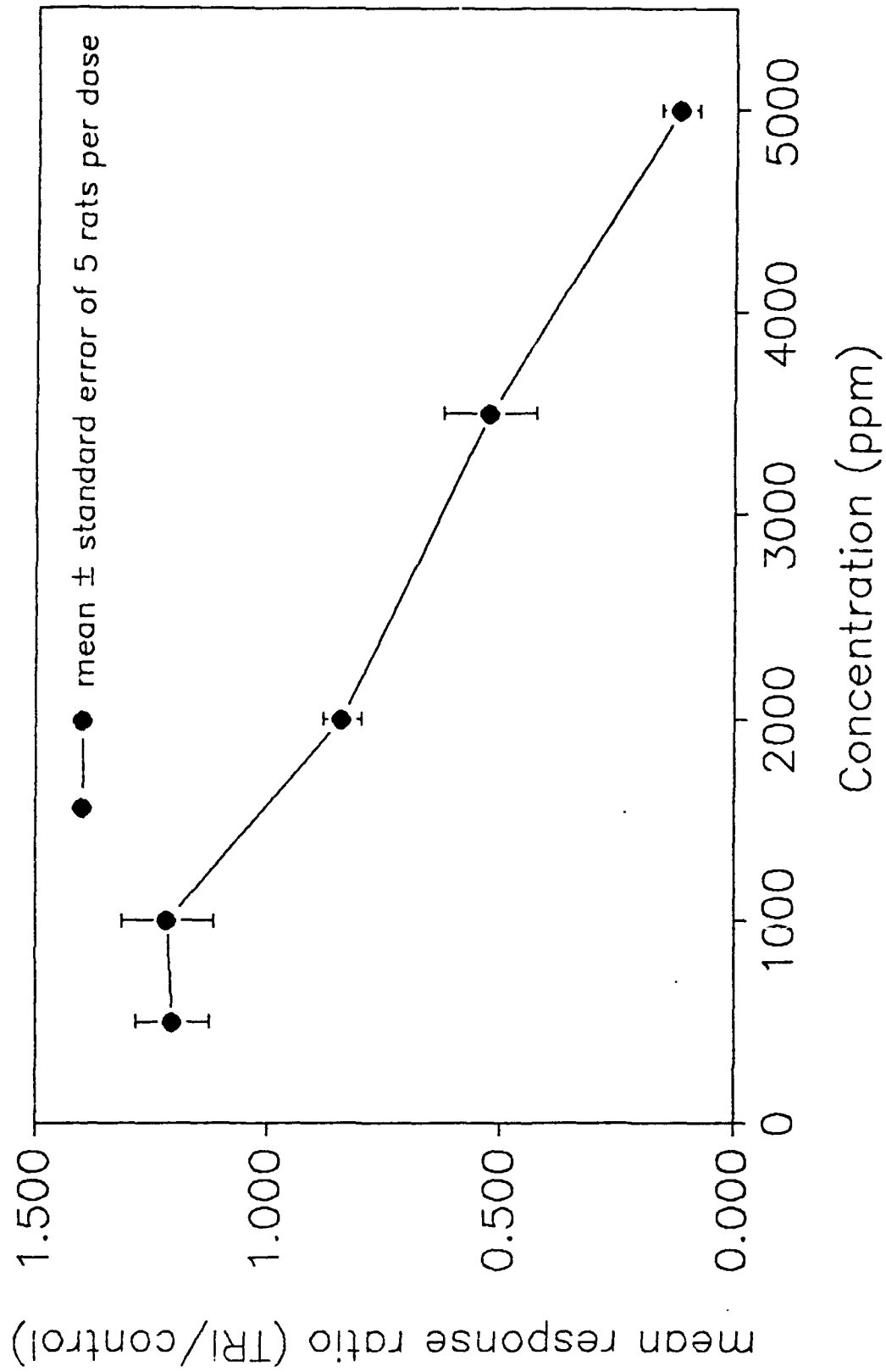
Responses During Inhalation of 3500 ppm IRI



Responses During Inhalation of 5000 ppm IKI



Dose-Response



Relationship of Brain Concentration of TRI to Operant Behavior

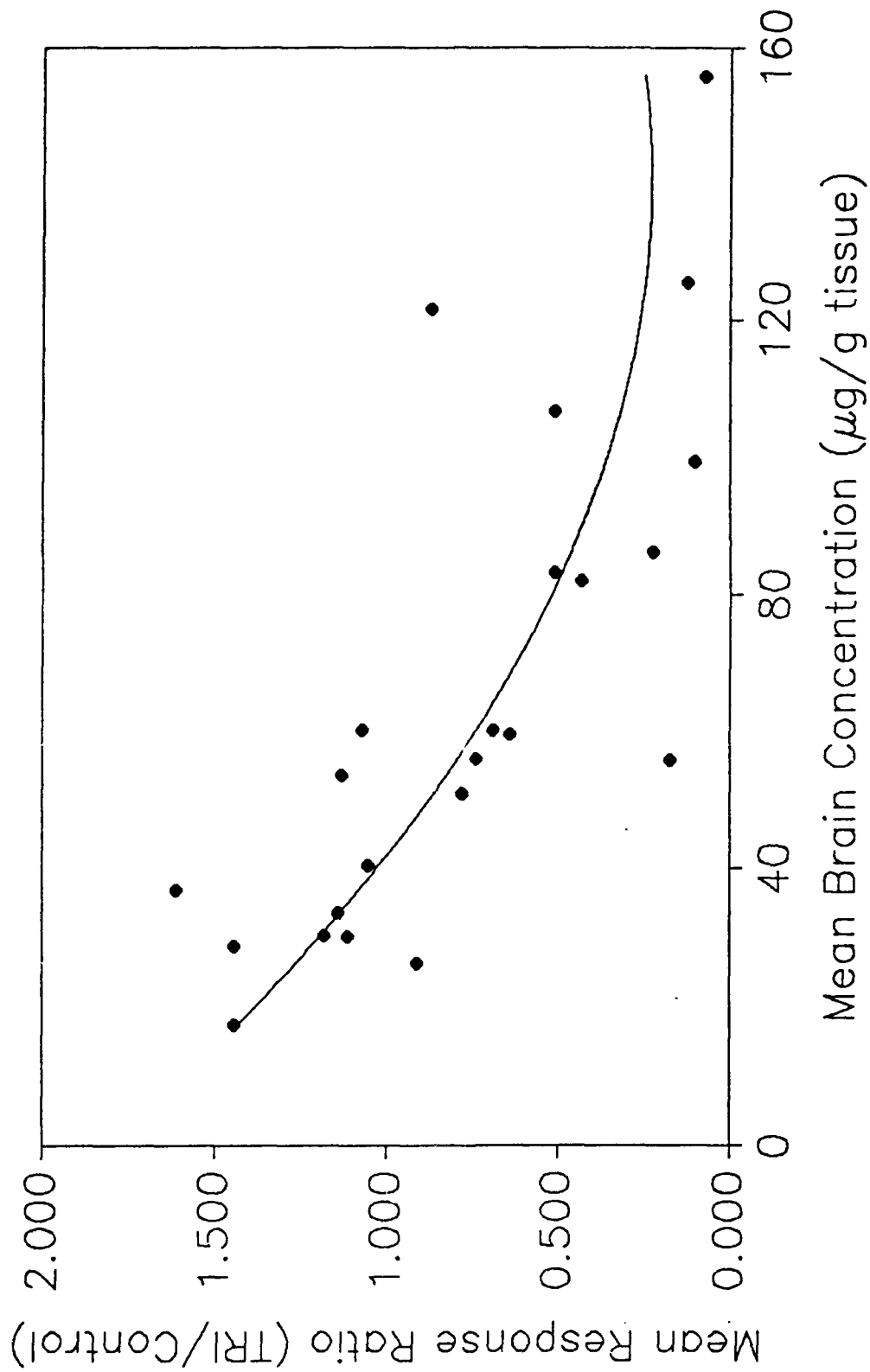


Figure 5

Relationship of Blood Concentration of TRI to Operant Behavior

